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CENTRAL TOXICOLOGY LABORATORY

Document Title
POLYMERIC MDI, 6-HOUR ACUTE INHALATION TOXICITY STUDY IN RATS WITH POST-EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS, WITH COVER LETTER DATED 3/15/1999

Chemical Category
BENZENE 1,1'-METHYLENEBIS(ISOCYANATO- (26447-40-5)

A 02

OFFICE OF TOXIC SUBSTANCES REV. 7/27/82

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March 15, 1999

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Attn: 8(d) HEALTH 7 SAFETY STUDY REPORTING RULE
(REPORTING)

Dear Sir or Madam:

We herewith submit a copy of the following recently completed health and safety study.

"POLYMERIC MDI: 6-HOUR ACUTE INHALATION TOXICITY STUDY IN RATS WITH POST_EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS."

Name of Chemical Substance:	benzene 1, 1'-METHYLENEBIS[ISOCYANATO-
Common name:	generic MDI
Chemical Abstracts Service Number:	26447-40-5
Abbreviation:	MDI

Authors:

N.J. Rattray
Central Toxicology Laboratory
Alderly Park, Macclesfield
Cheshire, UK

The International Isocyanate Institute (III) project identification number 11335 (Project 151) has been marked on the title page of the report. Please refer to the III identification number in any communication regarding this study. The enclosed report does not contain any Confidential Business Information.

This study was sponsored by the International Isocyanate Institute on behalf of the following:

The Dow Chemical Company
Bayer Corporation
BASF Corporation
ICI Americas, Inc.
Lyondell Chemical Company

CONTAINS NO CBI

Very truly yours,

M.J. Blankenship
Managing Director

Enclosure: Study

A 04

III Project 151

III ref. ---------

Polymeric MDI: 6 Hour Acute Inhalation Toxicity Study in Rats with Post-Exposure Observation Periods up to 30 Days

Author

N J Rattray
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March 199

Number of pages:

A 05

III Project 151

Supplementary information relating to III ref. ..... for III membership

SUMMARY

Groups of ten female rats were exposed nose-only for a single six hour period to aerosolised polymeric MDI at target concentrations of 10, 30 or 100mg/m^3. Test atmospheres were analysed for particulate concentration and Polymeric MDI. Following exposure, the animals were retained without treatment for periods of 1, 3, 10 or 30 days. Clinical observations and bodyweights were recorded and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem*. Lung lavage was carried out on selected animals at specified time points, listed tissues were examined from selected animals and lungs were weighed. 'S' phase analysis was carried out on selected animals at specified points.

Compound-related changes were seen at all exposure levels and the incidence and severity of the changes increased with exposure level. Clinical changes indicative of irritancy were present in all animals. Bodyweights of animals exposed to 90.1 or 27mg/m^3 were reduced by approximately 12 and 9% respectively, compared to their initial weights on day 2 of the study. Bodyweights of animals exposed to 9.9mg/m^3 were not significantly different from their initial values on day 2 of the study. There was a dose-related increase in lung weight in all animals exposed to polymeric MDI. There was a dose related increase in cellular exudate, and an increase in associated enzyme activities and protein. 'S' phase analysis increased in cell proliferation within the teminal bronchioles and centro-acinar alveoli. Examination of the lungs by light and electron microscopy showed clear evidence of a dose-related increase in Type II cell hyperplasia and levels of surfactant both in the lung and as lamellar bodies within the Type II cells. All parameters had returned to normal by the end of the study.

It was concluded that nose-only exposure for 6 hours to polymeric MDI at concentrations of 9.9, 27 or 90.1mg/m^3 resulted in effects at all concentrations. These included clinical changes, increased excretion of surfactant, increases in cellular exudates and related enzyme activities, a Type II cell hyperplasia and increases in the numbers and size of lamellar bodies within the Type II cells. All animals showed full recovery by day 31 following exposure.

Circulation list:

The following members have been sent full copies of the report:

Scientific Committee

Mr J H Chapman
Dr P Davies
Mr T Kosawa
Dr J P Lyon
Dr M Mann
Mr I Matsumura
Dr B Reeve
Mr J-C Ryou

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Dr C Bastian
Dr L Bloemen
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A 07

III Project 15

11335

Volume 1

CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

REPORT NO: CTL/P/5914

POLYMERIC MDI: 6-HOUR ACUTE INHALATION TOXICITY STUDY IN RATS WITH POST-EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

A 08

CE. TRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

REPORT NO: CTL/P/5914

POLYMERIC MDI: 6-HOUR ACUTE INHALATION TOXICITY STUDY IN RATS WITH POST-EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

STUDY DETAILS

Sponsor:	International Isocyanate Institute Inc. International Isocyanate Institute Scientific Office, Floor 9, Bridgewater House, Manchester, M1 6LT, UK
Sponsor Reference:	C05737
CTL Test Substance Reference Number:	Y00122/021
CTL Study Number:	HR2318

AUTHOR

N J Rattray

DATE OF ISSUE

15 February 1999

STATEMENT OF DATA CONFIDENTIALITY CLAIM

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO INTERNATIONAL ISOCYANATES INSTITUTE Inc.

It should not be disclosed in any form to an outside party, nor should information contained herein be used by a registration authority to support registration of this product or any other product without the written permission of International Isocyanate Institute Inc..

STATEMENT OF GLP COMPLIANCE AND AUTHENTICATION

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

The study was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1997) except for the deviation listed below. These Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

The OECD international standards are acceptable to the United States Environmental Protection Agency and this study, therefore, satisfies the requirements of 40 CFR Part 160 and 40 CFR Part 792.

The following GLP deviation is considered not to affect the integrity of the study or the validity of the conclusions drawn:

(i) the purity and stability of the test substance have not been reported.

N J Rattray — 15 February 1999
Study Director — Date

QUALITY ASSURANCE STATEMENT

In accordance with CTL policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Audit/Inspection	Date of QA Report
10 Nov 97	Protocol	11 Nov 97
17 Nov 97	Atmosphere generation, exposure, atmosphere collection	17 Nov 97
18-19 Nov 97	BrdU preparation, minipump implantation	19 Nov 97
26 Nov 97	Lung lavage	26 Nov 97
27 Nov 97	Post mortem	27 Nov 97
05 Dec 97	Bodyweights, clinical observations	05 Dec 97
24 Jun 98	Draft report	09 Jul 98
11 Feb 99	Final report review	15 Feb 99

In addition, inspections associated with this type of study were made as follows:

29 Oct 97	Analysis	29 Oct 97

The clinical pathology contribution has been audited by ZENECA Pharmaceuticals Quality Assurance as follows:

17 Dec 97		17 Dec 97
22 Jan 98		22 Jan 98
25 Mar 98		25 Mar 98

Facilities and process based procedures associated with this type of study were inspected in accordance with QA Standard Operating Procedures.

So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, HR2318.

I H Mills ....Irene H. Mills.... 15 February 1999

(CTL Quality Assurance Unit)

STUDY CONTRIBUTORS

The following contributed to this report in the capacities indicated:

Name	Title
N J Rattray	Study Director
I Pate	Statistician
N J Rattray	Author
S Millward	Study Technician
M Crawley	Chemical Analyst
C Bowling	Electron Microscopist
J Foster	Pathologist
P M Hext	Study Reviewer
S J Duffell	Clinical Pathologist
K P Jackson	Principal Investigator Zeneca Pharmaceuticals Alderley Park Macclesfield Cheshire UK

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Volume 2

CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

REPORT NO: CTL/P/5914

PHOTOGRAPHIC SUPPLEMENT TO POYMERIC MDI: 6 HOUR ACUTE INHALATION TOXICITY STUDY RATS WITH POST EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

PHOTOGRAPHS OF MAIN ULTRASTRUCTURAL FINDINGS

B 03

CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

REPORT NO: CTL/P/5914

PHOTOGRAPHIC SUPPLEMENT TO POYMERIC MDI: 6 HOUR ACUTE INHALATION TOXICITY STUDY RATS WITH POST EXPOSURE OBSERVATION PERIODS UP TO 30 DAYS

PHOTOGRAPHS OF MAIN ULTRASTRUCTURAL FINDINGS

STUDY DETAILS

Sponsor:	International Isocyanate Institute Inc. International Isocyanate Institute Scientific Office, Floor 9, Bridgewater House, Manchester, M1 6LT, UK
Sponsor Reference:	C05737
CTL Test Substance Reference Number:	Y00122/021
CTL Study Number:	HR2318

AUTHOR

N J Rattray

DATE OF ISSUE: 15 February 1999

B 04

I, the undersigned declare that this supplement constitutes a true record of the results obtained in the above study.

N.Rattray Nuchla J Rattray..... Date 15 February 1999

(Study Director)

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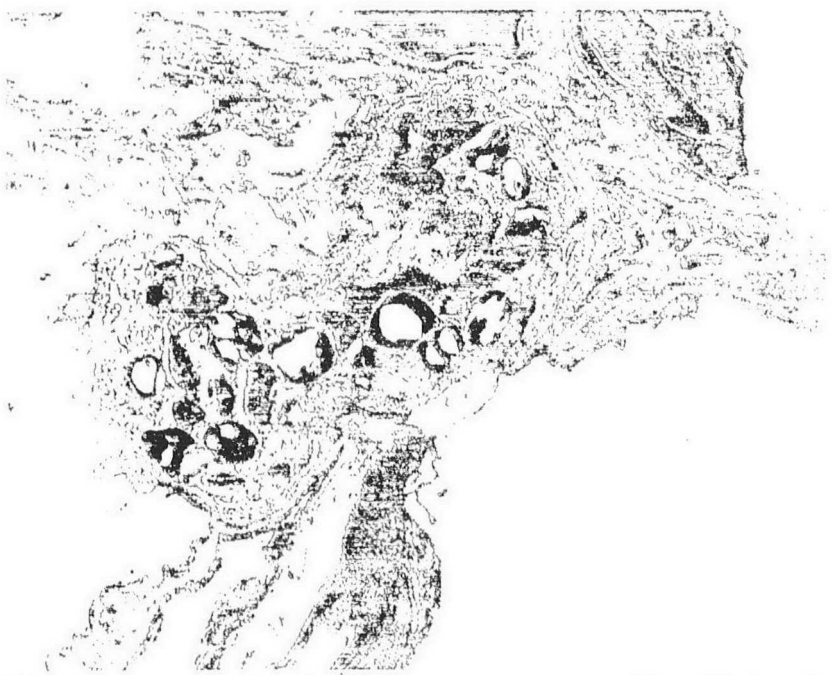
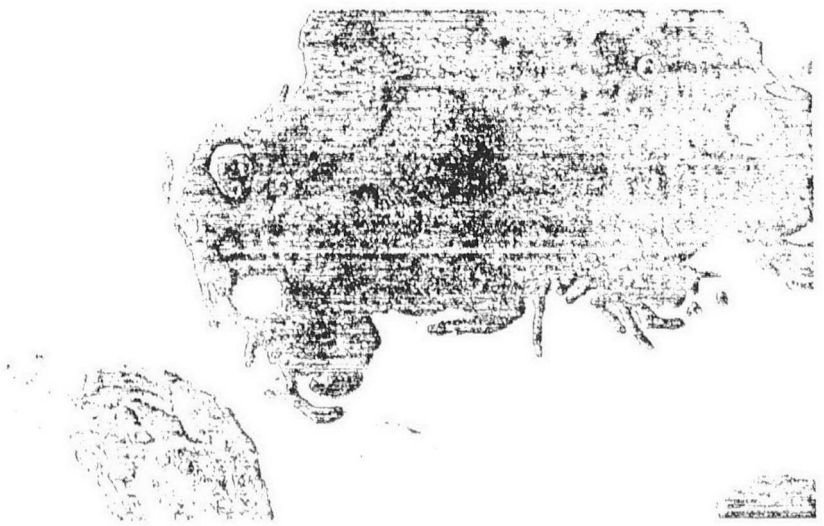
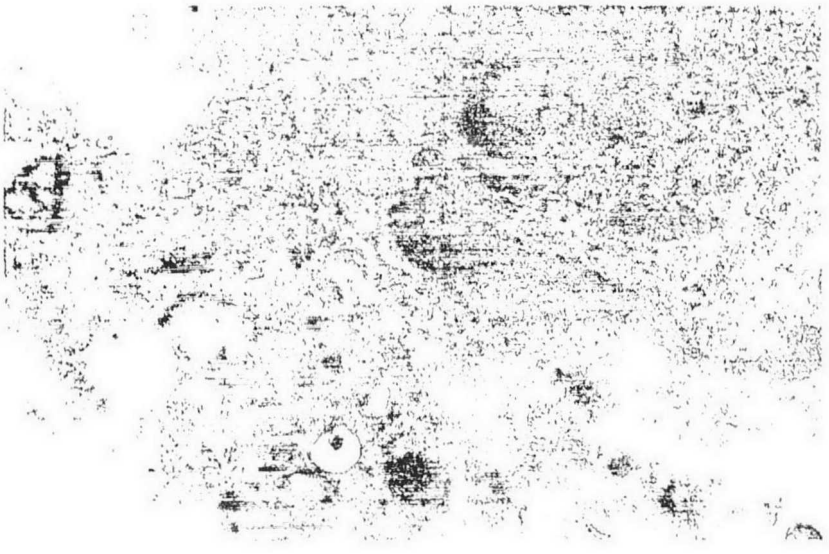
PHOTOGRAPH 28 Animal 319, 10mg/m^3 30 days post exposure. Pulmonary macrophage. 20



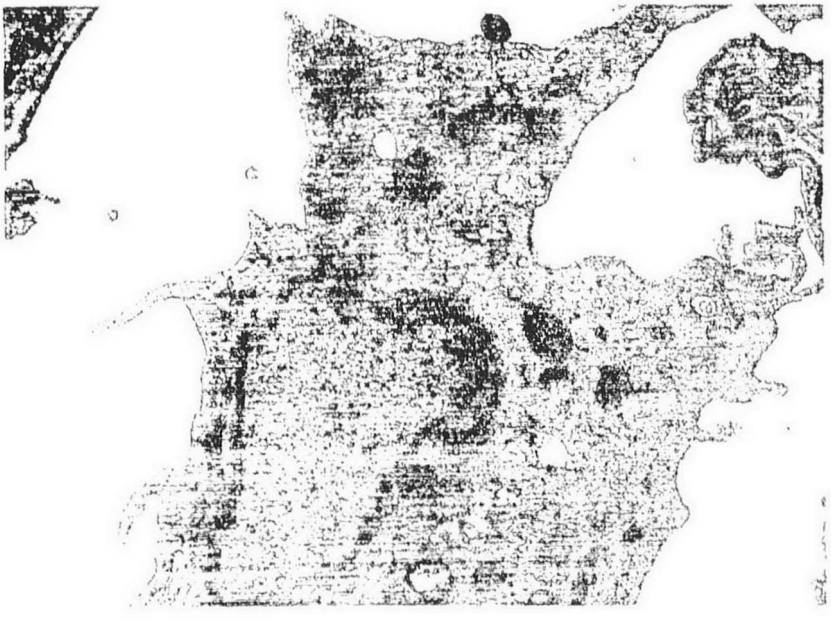
PHOTOGRAPH 1 Animal 9, 0mg/m^3 1 day post exposure. Type II alveolar cell. Magnification x 12,000



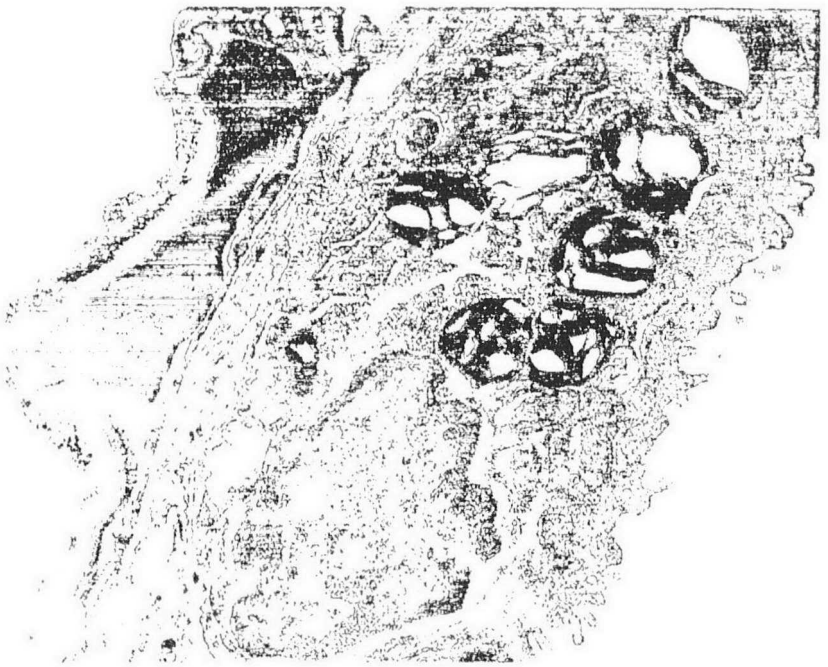
PHOTOGRAPH 2 Animal 10, 0mg/m^3 1 day post exposure. Pulmonary macrophage. Magnification x 15,000



PHOTOGRAPH 3 Animal 10, 0mg/m^3 1 day post exposure. Pulmonary macrophage. Magnification x 36,000

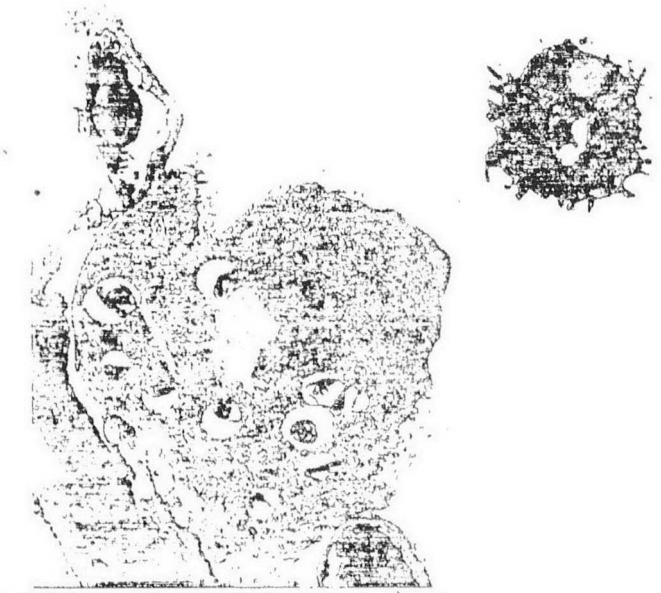


PHOTOGRAPH 4 Animal 209, 0mg/m^3 3 days post exposure. Pulmonary macrophage. Magnification x 30,000



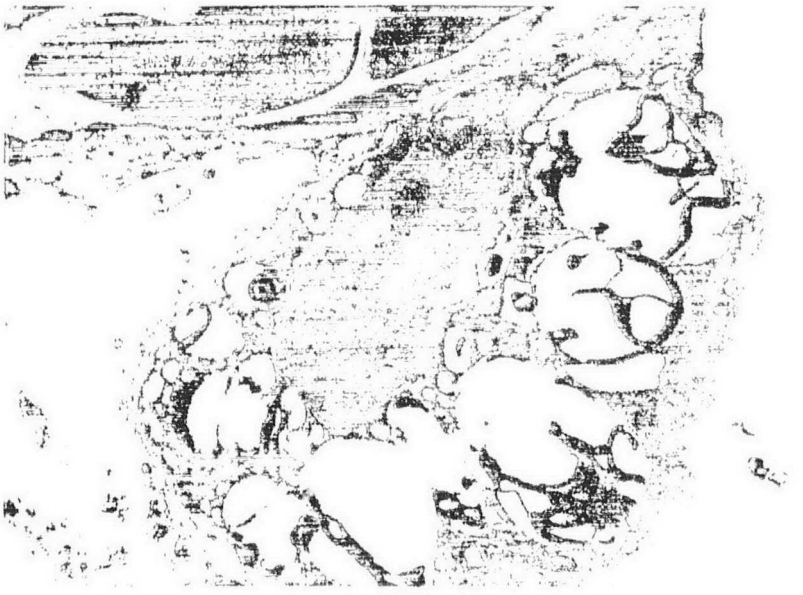
PHOTOGRAPH 5 Animal 210, 0mg/m^3 10 days post exposure. Type II alveolar cell.

Magnification x 30,000



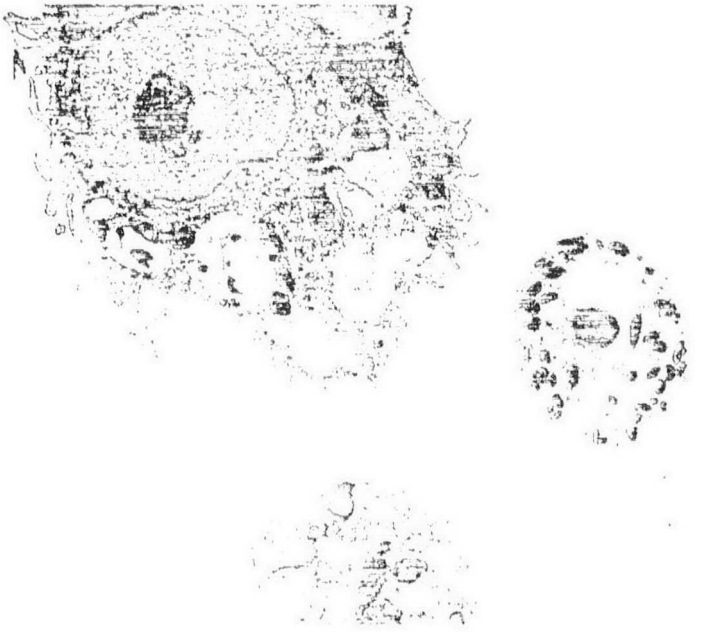
PHOTOGRAPH 6 Animal 36, 90mg/m^3 1 day post exposure. Type II alveolar cell with adjacent pulmonary macrophage free in the lumen.

Magnification x 9,000



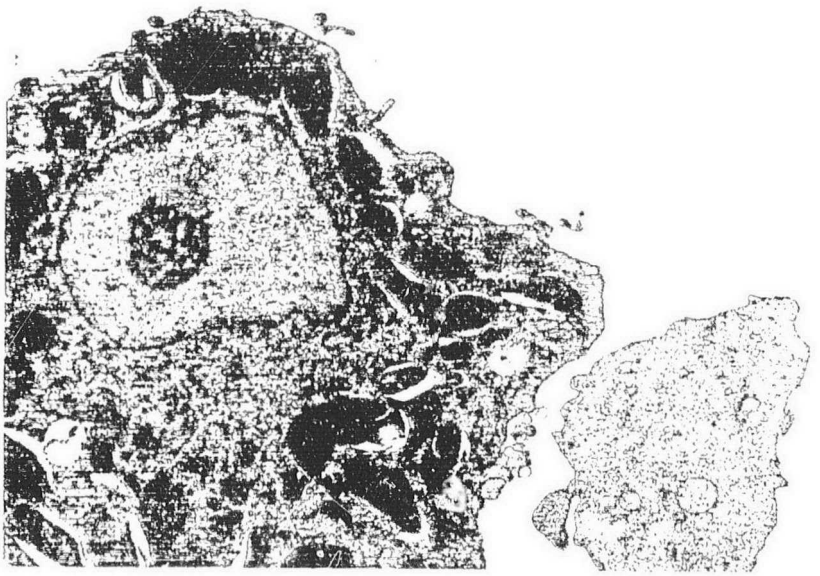
PHOTOGRAPH 7 Animal 37, 90mg/m^3 1 day post exposure. Type II cell showing increased lamellar size.

Magnification x 19,000



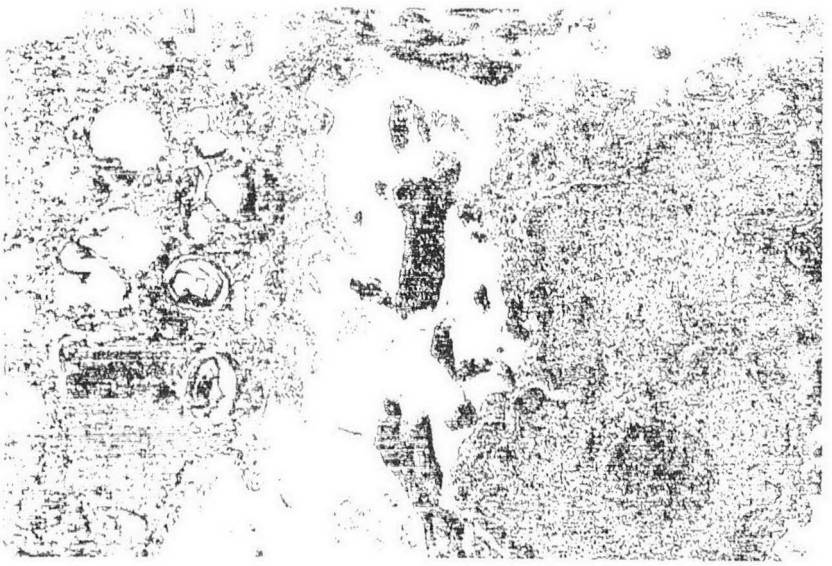
PHOTOGRAPH 8 Animal 36, 90mg/m^3 1 day post exposure. Pulmonary macrophage showing amorphous inclusions.

Magnification x 9,000



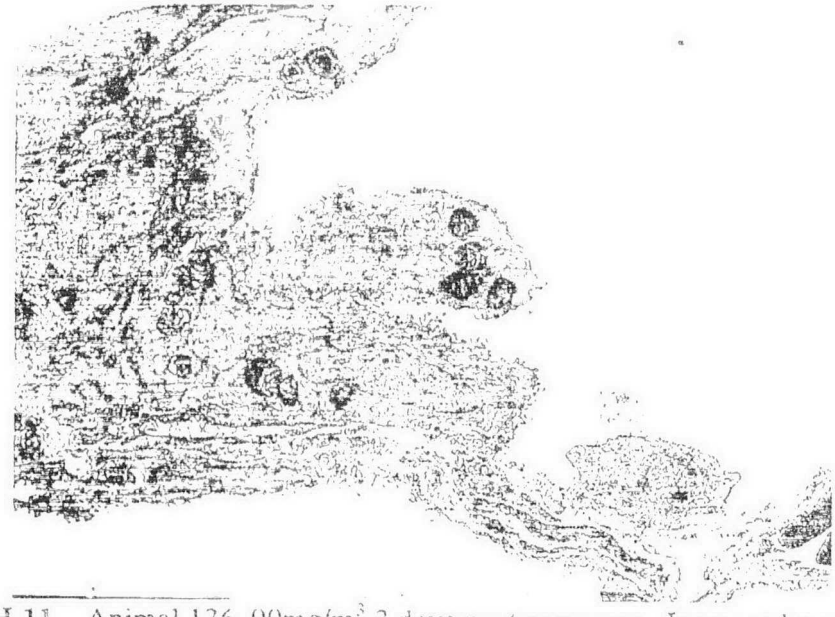
PHOTOGRAPH 9 Animal 37, 90mg/m^3 1 day post exposure. Macrophage with amorphous material.

Magnification x 15,000



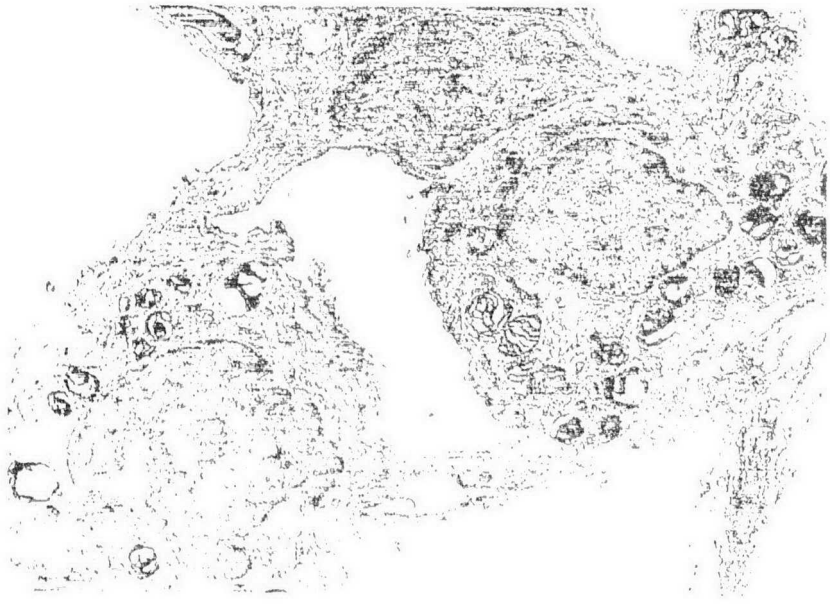
PHOTOGRAPH 10 Animal 29, 27mg/m^3 1 day post exposure. Pulmonary macrophage and luminal surfactant.

Magnification x 24,000



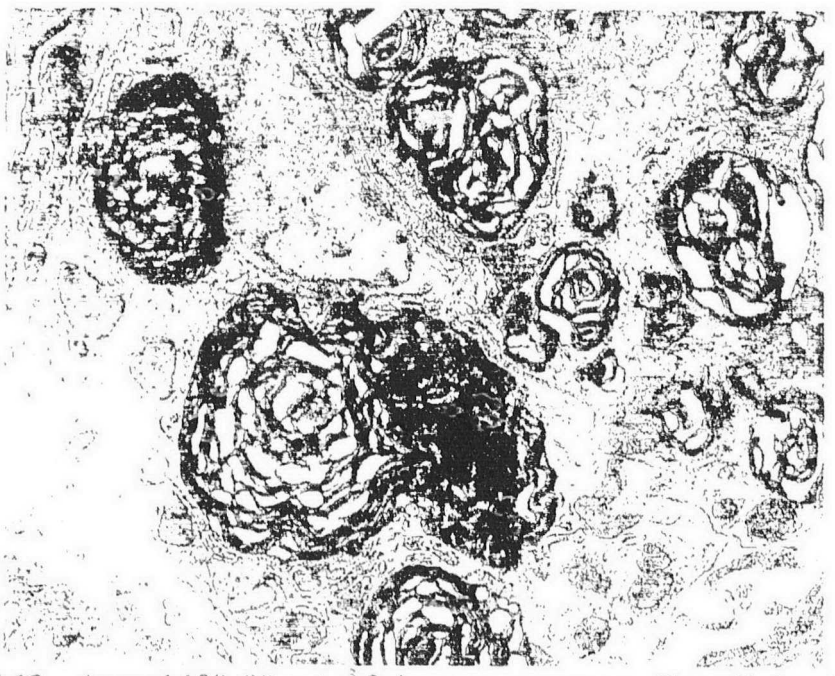
PHOTOGRAPH 11 Animal 136, 90mg/m^3 3 days post exposure. Increased numbers of Type II cells.

Magnification x 6,600



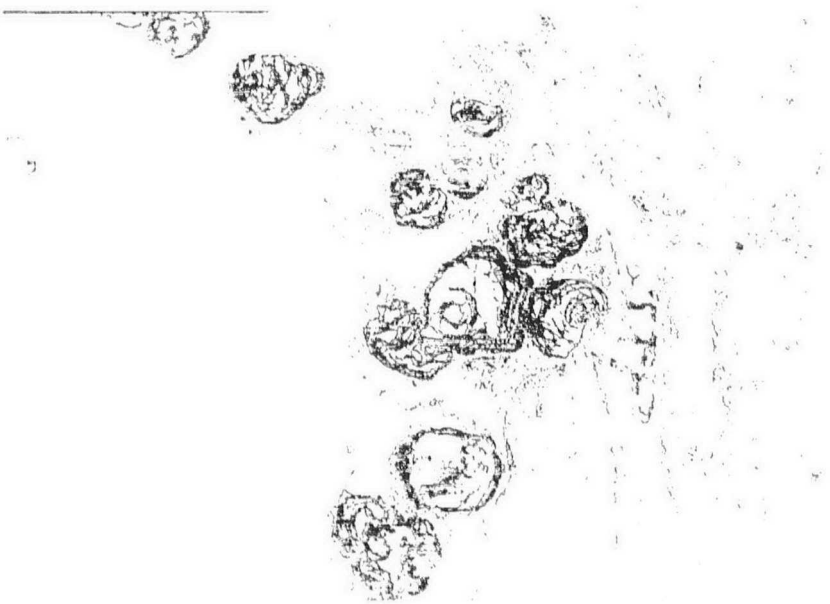
PHOTOGRAPH 12 Animal 118, 9.9mg/m^3 3 days post exposure. Type II alveolar cell.

Magnification x 12,000



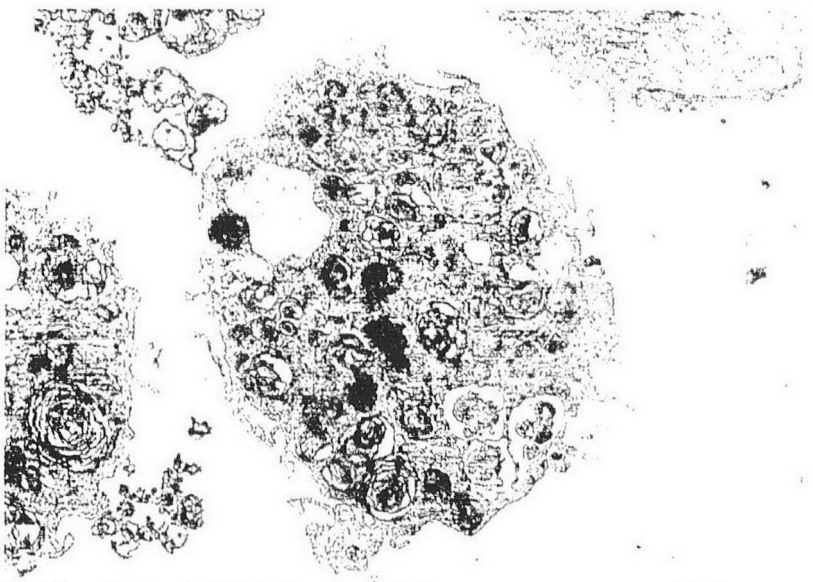
PHOTOGRAPH 13 Animal 138, 90mg/m^3 3 days post exposure. Type II alveolar cell showing enlarged lamellar bodies.

Magnification x 36,000



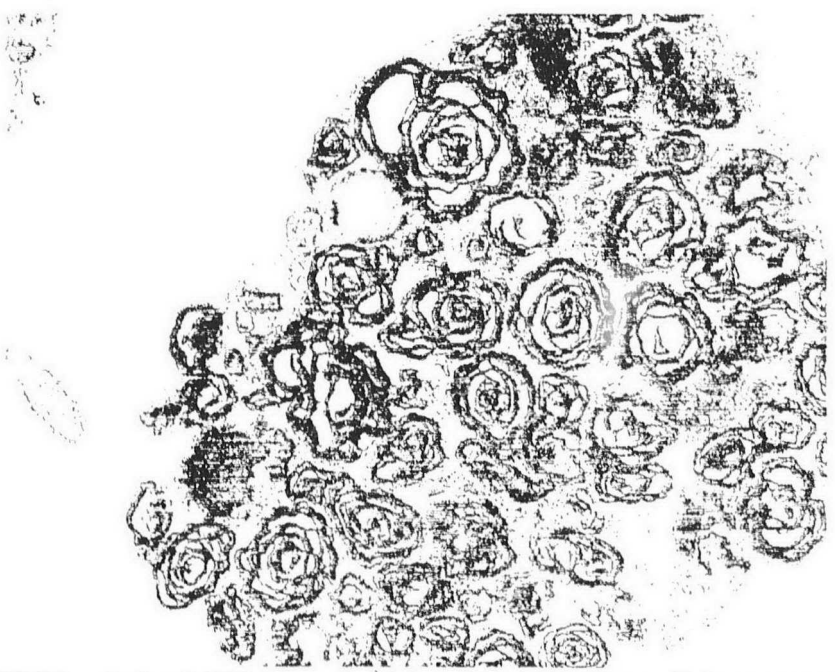
PHOTOGRAPH 14 Animal 137, 90mg/m^3 3 days post exposure. Type II alveolar cell showing enlarged lamellar bodies.

Magnification x 15,000



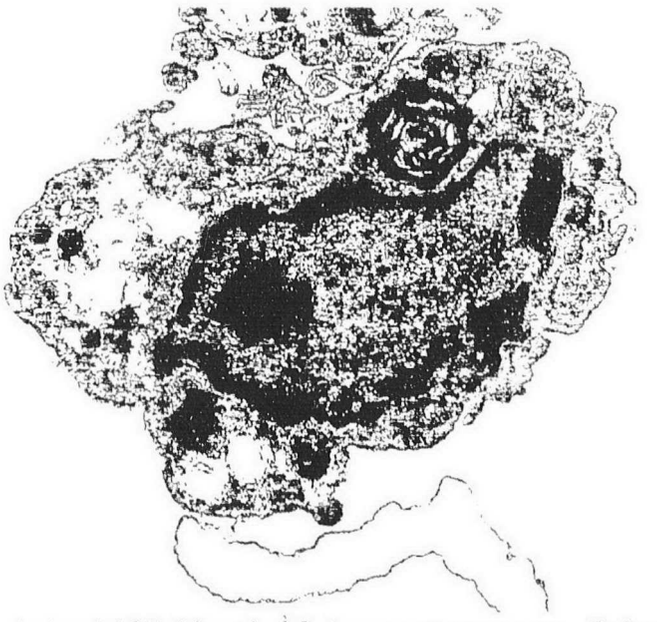
PHOTOGRAPH 15 Animal 139, 90mg/m^3 3 days post exposure. Pulmonary macrophage with ingested lamellar bodies and surfactant.

Magnification x 15,000



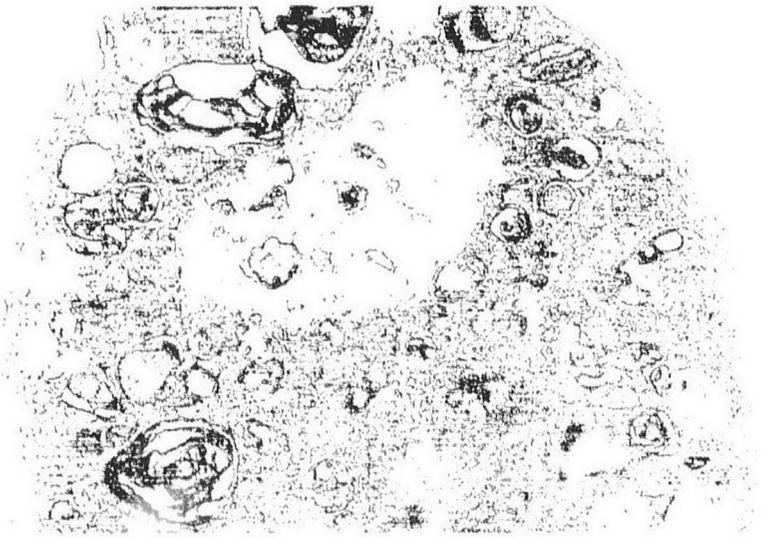
PHOTOGRAPH 16 Animal 137, 90mg/m^3 3days post exposure. Pulmonary macrophage (as above) with numerous lamellar inclusions..

Magnification x 24,000



PHOTOGRAPH 17 Animal 137, 90mg/m^3 3 days post exposure. Pulmonary macrophage with crystalline inclusion.

Magnification x 30,000

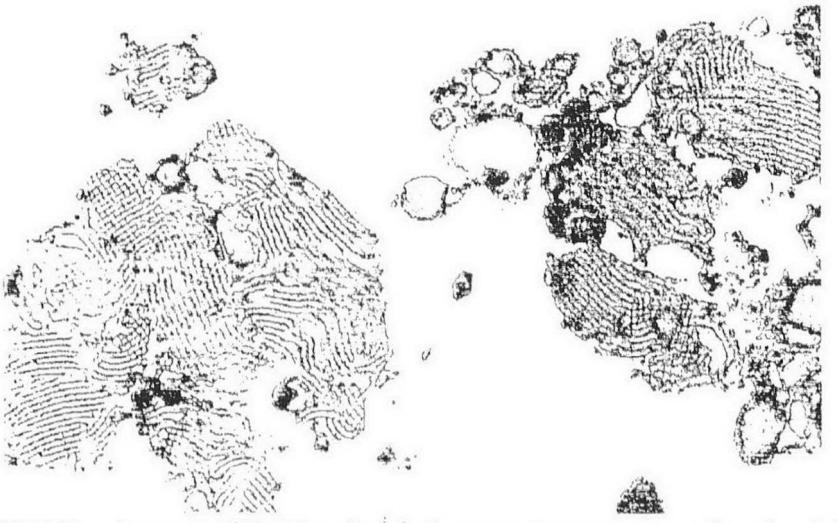


PHOTOGRAPH 18 Animal 130, 27mg/m^3 3 days post exposure. Pulmonary macrophage ingested lamellar bodies.

Magnification x 24,000

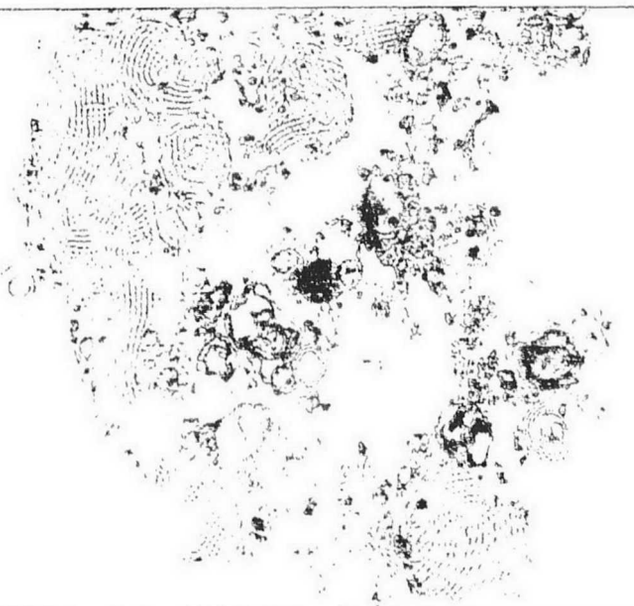
PHOTOGRAPH 19 Animal 118, 9.9mg/m^3 3 days post exposure. Type II cell with alveolar macrophage.

Magnification x 12,000



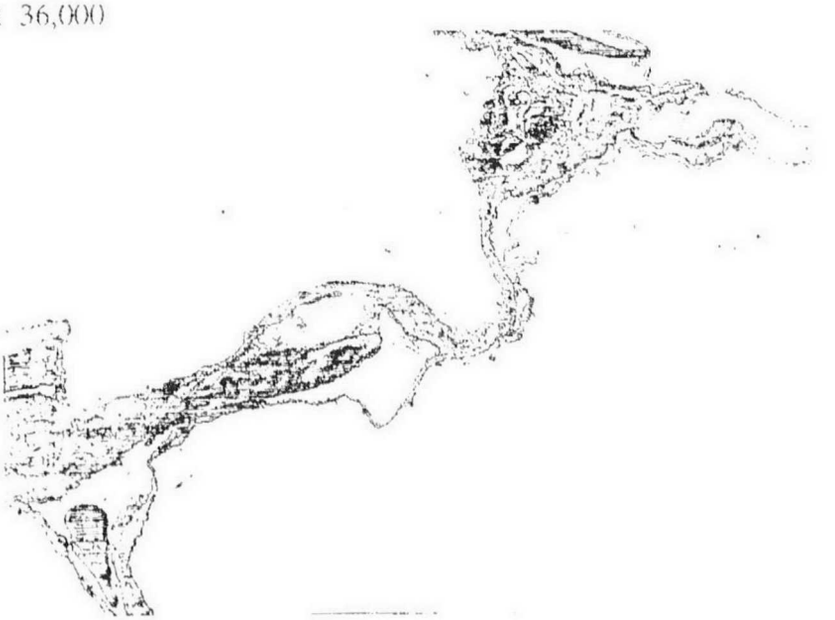
PHOTOGRAPH 20 Animal 137, 90mg/m^3 3 days post exposure. Alveolar debris including crystalline hypophase surfactant.

Magnification x 36,000



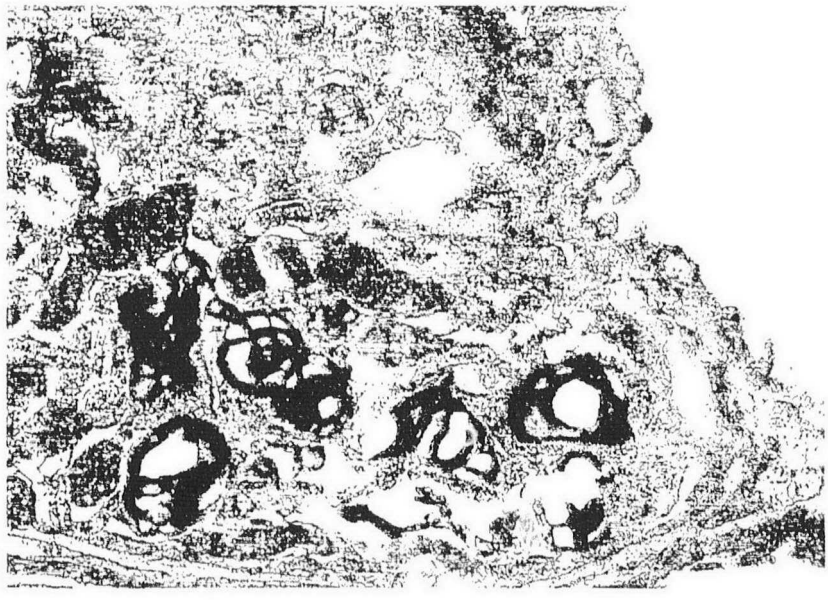
PHOTOGRAPH 21 Animal 139, 90mg/m^3 3 days post exposure. Alveolar debris including crystalline hypophase surfactant.

Magnification x 36,000



PHOTOGRAPH 22 Animal 237, 90mg/m^3 10 days post exposure. Type II alveolar cell.

Magnification x 6,000



PHOTOGRAPH 23 Animal 237, 90mg/m^3 10 days post exposure. Type II alveolar cell. Magnification x 36,000

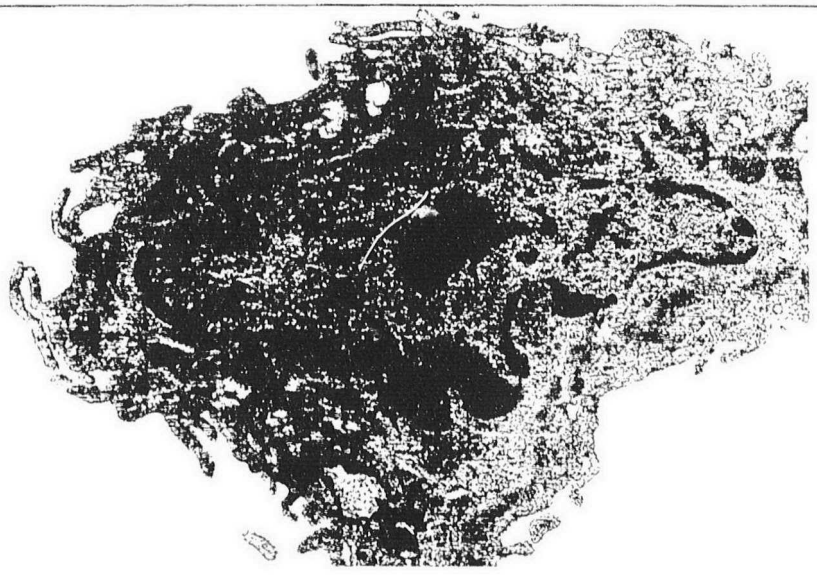
PHOTOGRAPH 24 Animal 237, 90mg/m^3 10 days post exposure. Type II alveolar cell. Magnification x 30,000

PHOTOGRAPH 25 Animal 240, 90mg/m^3 10 days post exposure. Pulmonary macrophage.

Magnification x 21,000

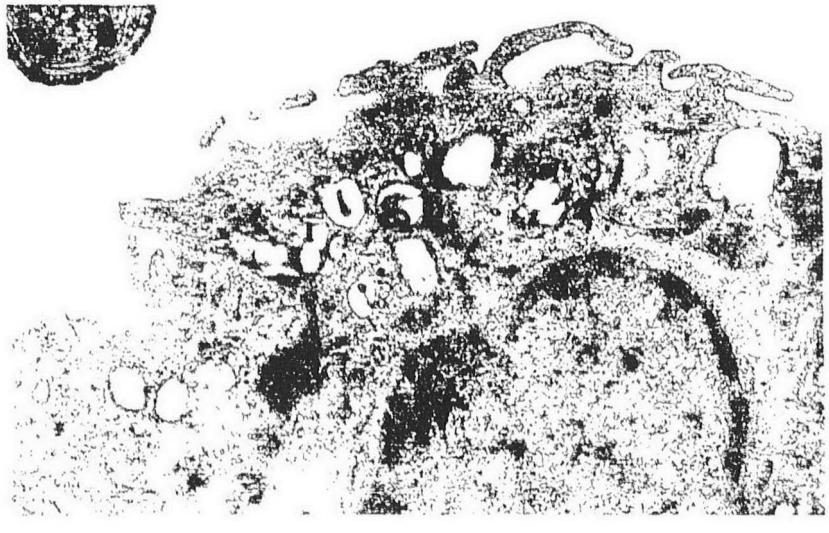
PHOTOGRAPH 26 Animal 338, 90mg/m^3 30 days post exposure. Type II alveolar cell.

Magnification x 9,000



PHOTOGRAPH 27 Animal 328, 27mg/m^3 30 days post exposure. Pulmonary macrophage.

Magnification x 30,000



PHOTOGRAPH 28 Animal 319, 10mg/m^3 30 days post exposure. Pulmonary macrophage.

Magnification x 36,000

1. SUMMARY

1.1 Study design

Groups of ten female Alpk:AP$_f$SD (Wistar-derived) rats were exposed nose-only for a single six-hour period to aerosolised polymeric MDI at target concentrations of 10, 30 or 100mg/m^3. Test atmospheres were analysed for particulate concentration and Polymeric MDI. The particle size distribution of each test atmosphere was analysed twice during the exposure period. Following exposure, the animals were retained without treatment for periods of 1, 3, 10 or 30 days. Clinical observations and bodyweights were recorded and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem*. Lung lavage was carried out on selected animals at specified time points, listed tissues were examined from selected animals and lungs were weighed. 'S' phase analysis was carried out on selected animals at specified time points.

1.2 Results

The achieved test atmospheres had the following characteristics:

Target Concentration mg/m^3	Particulate Concentration mg/m^3	MMAD*	GSD$^+$
10	9.9	1.09	1.48
30	27	1.25	1.54
100	90.1	0.96	1.6

* Mass Median Aerodynamic Diameter
$^+$ Geometric Standard Deviation

Compound-related changes were seen at all exposure levels and the incidence and severity of the changes increased with exposure level. Clinical changes indicative of irritancy were present in all animals. Bodyweights of animals exposed to 90.1 or 27mg/m^3 were reduced by approximately 12 and 9% respectively, compared to their initial weights on day 2 of the study. Bodyweights of animals exposed to 9.9mg/m^3 were not significantly different from their initial

values on day 2 of the study. There was a dose-related increase in lung weight in all animals exposed to polymeric MDI. There was a dose-related increase in cellular exudate, and an increase in associated enzyme activities. There was a marked dose-related increase in protein exudate. 'S' phase analysis indicated increased cell proliferation within the terminal bronchioles and centro-acinar alveoli. Examination of the lungs by light and electron microscopy showed clear evidence of a dose-related increase in Type II cell hyperplasia and levels of surfactant both in the lung and as lamellar bodies within the Type II cells. All parameters had returned to normal by the end of the study.

1.3 Conclusion

Nose-only exposure for 6 hours to polymeric MDI at concentrations of 9.9, 27 or 90.1mg/m^3 resulted in effects at all concentrations. These included clinical changes, increased excretion of surfactant, increases in cellular exudates and related enzyme activities, a Type II cell hyperplasia and increases in the numbers and size of lamellar bodies within the Type II cells. These changes showed a dose-related increase in severity and incidence. There was an adverse effect on bodyweights in animals at 27 and 90.1mg/m^3 but at 9.9mg/m^3 bodyweights were not affected.

All animals showed full recovery by day 31 of the study.

2. INTRODUCTION

2.1 Purpose

The purpose of this study was to investigate the effect on the rat lung of a single 6 hour exposure to polymeric MDI aerosol after 1, 3, 10 and 30 days following exposure.

2.2 Regulatory guidelines

This study was not conducted to any specific regulatory guidelines.

2.3 Justification for test system selection

The Alpk:APfSD strain of rat was used because of the substantial background data available for this strain, in this Laboratory, relating to studies of this type. Inhalation was the required route for administration of polymeric MDI for this study.

2.4 Selection of atmospheric concentrations

Atmospheric concentrations of 10, 30 or 100mg^3, were selected by the Sponsor on the basis of the known inhalation toxicity of polymeric MDI.

2.5 Study dates

The study was initiated on 4 November 1997. The experimental phase started on 10 November 1997. Exposures were carried out as follows: procedure groups 3 and 4 on 17 November 1997, procedure group 1 on 25 November 1997 and procedure group 2 on 2 December 1997. The study was completed on 26 March 1998.

2.6 Data storage

An original report, all raw data, samples and specimens pertaining to this study, with the exception of the lung lavage raw data, are retained in the Archives, Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK. Lung lavage (clinical chemistry assessments) raw data are stored in the Safety of Medicine Archives, Zeneca Pharmaceuticals, Alderley Park.

3. TEST SUBSTANCE

Name:	Polymeric MDI (Suprasec 5005) Supplied by ICI Polyurethanes, Hill House Works, Lancs., UK
CAS No.:	9016-87-9
Colour:	Dark brown
Physical state:	Liquid
Batch reference number:	LAB Ref 982
CTL test substance reference number:	Y00122/021
Stability:	The stability of the test substance was re-characterised at each analysis point during the experimental phase of the test by the chemical analyst.
Storage conditions:	Ambient temperature in the dark.

The sample was tested as supplied.

The purity and stability of the test substance are the responsibility of the Sponsor.

4. EXPERIMENTAL PROCEDURES

4.1 Atmosphere generation

4.1.1 Trial generation

Trial generations were carried out prior to the start of the study in order to:

i) determine the appropriate generation system and conditions

ii) determine that appropriate concentrations could be achieved

iii) obtain data on the aerodynamic particle size of the atmospheres generated

iv) determine an appropriate method of analysis of polymeric MDI in the particulate phase of the test atmosphere.

4.1.2 Generation conditions

Each test atmosphere was generated using a glass concentric-jet atomiser and cyclone. The test substance was pumped to the atomiser using a peristaltic pump. Clean, dry air (dried and filtered using equipment supplied by Atlas-Copco, Sweden) was passed through the atomiser at nominal flow rates of 16, 14 or 3.5-16*l*/minute at for the 10, 30 or 100mg/m^3 concentrations respectively and carried the atmosphere to each of the exposure chambers (internal volume of 27.6 litres). Diluting air was added directly to the exposure chambers at a flow rate of 25-30, 25-30 or 10-30*l*/minute to achieve the target concentrations to give a minimum total chamber flow rate of 41, 39 or 26*l*/minute respectively for the 10, 30 or 100mg/m^3 concentrations. Air flows were monitored and recorded at approximately 30 minute intervals using variable area flowmeters and were altered as necessary to maintain the target concentrations.

4.2 Atmosphere sampling and analysis

4.2.1 Particulate concentrations

The particulate concentration of each test atmosphere, close to the animals' breathing zone, was measured gravimetrically at least three times during exposure. This was done by drawing each test atmosphere, at a known flow rate for a known time, through a 25mm diameter, Institute of Occupational Medicine (IOM) sampler SKC Ltd., Blandford Forum, Dorset, UK. Prior to sampling, filters for the sampler were impregnated with 1-(2-methoxyphenyl) piperazine (1,2-MP) reagent. The filter was weighed before and after the sample was taken. The concentration was calculated as follows:

$$\text{concentration (mg/}l\text{)} = \frac{\text{post wt - pre wt}}{\text{time (minutes) x airflow (}l\text{/min)}}$$

pre wt = weight of filter prior to sampling (mg)

post wt = weight of filter after sampling (mg)

4.2.2 Analysed atmospheric concentrations

The atmospheric concentration of polymeric MDI was determined for each exposure level by analysis of the material collected on the IOM filters. The analytical method is given in Appendix A.

4.2.3 Aerodynamic particle size

The aerodynamic particle size of each test atmosphere was measured once during exposure by means of a Marple Cascade Impactor (supplied by Schaefer Instruments Limited, Wantage, Oxon, UK) which aerodynamically separates airborne particles into pre-determined size ranges. Using a microcomputer, the data were transformed using a log/probit transformation and a linear regression derived from the cumulative data.

Using this regression line, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated. Definitions of particle size are given in Appendix B.

4.3 Experimental design

4.3.1 Animals

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	Rodent Breeding Unit , Zeneca Pharmaceuticals, Alderley Park Macclesfield, Cheshire.
Sex:	172 female
Specification.	Young adult (6-8 weeks)

4.3.2 Accommodation and husbandry

The rats were housed 5 per cage in multiple rat racks suitable for animals of this strain and the weight range expected during the course of the study.

The rats were transferred to clean cages and racks, as necessary, during the study.

The animal room was designed to give the environmental conditions shown as follows:

Temperature:	22±3°C
Relative humidity:	30-70%
Air:	At least 15 changes/hour.
Light cycle:	Artificial giving 12 hours light, 12 hours dark

Both temperature and relative humidity were measured and recorded daily values were within the specified ranges.

Diet (RM1) supplied by Special Diet Services Limited, Witham, Essex, UK and mains water, supplied by an automatic system were available *ad libitum*, except during exposure.

Each batch of diet is routinely analysed for composition and for the presence of contaminants. Water is also periodically analysed for the presence of contaminants. No contaminants were found to be present in the diet or water at levels considered to be capable of interfering with the purpose or outcome of the study. Certificates of analyses are retained in the CTL Archives.

4.3.3 Acclimatisation

The animals were housed under the experimental conditions for at least 1 week at CTL, prior to the start of the study

4.3.4 Animal randomisation and identification

The animals were allocated to the groups using the method shown in Appendix C. The individual animal numbers for each cage were determined by group and replicate and are shown in the rack plan (Appendix D). In addition, after acclimatisation, rats were familiarised to the restraint tubes prior to exposure on days -3, -2 and -1 for periods of 2, 4 and 6 hours respectively. Wherever possible, measurements were made in replicate order. Animals were individually identified with their allocation number by ear punching. Any animals not required for the study were discarded.

On the front of each cage of animals was a card identifying the contained animals by exposure concentration, group number, individual number, sex and study.

4.3.5 Atmospheric concentrations and exposure groups

The study consisted of four groups of forty female rats. The experimental numbers and the group to which the rats were assigned are shown in section 4.3.6.

4.3.6 Duration of exposures, retention periods and animal numbers

The animals were given a single exposure of 6 hours and retained for periods of up to 30 days. Following exposure animals were retained and killed at the time points specified in the following tables:

Single exposure and examination on the day following exposure (day 2).

Test group	Colour code	Concentration (mg/m^3)	Animal Nos for lung lavage	Animal Nos for pathology
1.1	blue	0	1-5	6-10
1.2	green	10	11-15	16-20
1.3	yellow	30	21-25	26-30
1.4	red	100	31-35	36-40

Nos = numbers

Single exposure and examination after 3 days post-exposure observation (day 4).

Test group	Colour code	Concentration (mg/m^3)	Animal Nos for lung lavage	Animal Nos for pathology
2.1	blue	0	101-105	106-110
2.2	green	10	111-115	116-120
2.3	yellow	30	121-125	126-130
2.4	red	100	131-135	136-140

Nos = numbers

Single exposure and examination after 10 days post-exposure observation (day 11).

Test group	Colour code	Concentration (mg/m^3)	Animal Nos for lung lavage	Animal Nos for pathology
3.1	blue	0	201-205	206-210
3.2	green	10	211-215	216-220
3.3	yellow	30	221-225	226-230
3.4	red	100	231-235	236-240

Nos = numbers

Single exposure and examination after 30 days post-exposure observation (day 31).

Test group	Colour code	Concentration (mg/m^3)	Animal Nos for lung lavage	Animal Nos for pathology
4.1	blue	0	301-305	306-310
4.2	green	10	311-315	316-320
4.3	yellow	30	321-325	326-330
4.4	red	100	331-325	336-340

Nos = numbers

4.3.7 Exposure system

The rats were exposed nose-only in restraining tubes supplied by Battelle, Geneva, Switzerland. These tubes were inserted into a PERSPEX exposure chamber (Appendix E) once the target concentration had been achieved and shown to be acceptably stable over approximately 30 minutes. Temperature and relative humidity within each chamber was measured at frequent intervals during exposure using a portable, digital temperature and relative humidity monitor. Within the chamber, the temperature ranged between 17.5 and 20.1°C and the relative humidity between 9 and 47%.

4.4 Clinical observations

Prior to the start of the study, all rats were examined to ensure that they were physically normal and exhibited normal activity. During exposure, they were observed frequently and, at the end of the 6 hour exposure period, each rat was given a detailed clinical examination. The animals were also subjected to detailed clinical observations, included the finding of no abnormalities detected, on day 1, daily to day 10, on days 15, 22, 29 and at scheduled termination.

4.5 Bodyweights

The bodyweight of each rat was recorded on days 1, 8, 15, 22, 29 and prior to scheduled termination.

4.6 BrdU labelling indices

Animals designated for 'S' phase analysis were implanted subcutaneously with osmotic Minipumps (Azlet model 2ML1) containing BrdU 7 days prior to scheduled termination.

4.7 Investigations *post mortem*

4.7.1 Termination

All animals were exsanguinated under terminal anaesthesia with euthetal.

4.7.2 Lung lavage

The lungs were lavaged *in situ* with 5-10ml of isotonic saline which, on withdrawal, was centrifuged and the supernatant decanted from the cell pellet. The supernatant from this first

lavage samples were submitted to, and analysed by, Safety of Medicines, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK. The lavage procedure was then repeated a further 5 times and these subsequent lavages were pooled and added to the initial cell pellet. The total volume was then centrifuged, the supernatant decanted from the cell pellet and discarded and the cell pellet was resuspended in 1ml of isotonic saline.

4.7.3 Cytological examination of lung lavage

The cell pellets derived from the centrifugation of the lung lavage fluid obtained from each animal were suspended in 1ml of phosphate buffered saline.

The cell count was carried out on a 100µl sample diluted to 1/10 with 900µl of isotonic saline prior to counting.

A 50µl quantity of the cell suspension (well mixed by gentle hand shaking) from each animal was loaded into a sample chamber of a *Cytospin 2* machine and spun for 10 minutes at 600rpm. The prepared slides were then fixed in methanol for 10 minutes and stained. A manual differential count was performed dividing the cell population into four cell types: alveolar macrophages, polymorphonuclear leucocytes, eosinophils and lymphocytes/other cell types. This latter group comprised mononuclear cells lacking plentiful cell cytoplasm as seen in alveolar macrophages.

4.7.4 Examination of lung lavage fluid

The following were measured in the supernatant fluid from the first lavage sample:

total protein	lactate dehydrogenase
alkaline phosphatase	N-acetyl glucosaminidase

4.7.5 Lung weights

Lungs (with trachea attached but larynx removed) were weighed from animals designated for pathology.

4.7.6 Macroscopic examination

All animals designated for pathology were subjected to an examination *post mortem*. This involved an external observation and a careful examination of all thoracic and abdominal viscera, brain and cranial cavity.

4.7.7 Tissue submission

The following tissues were taken from all animals designated for pathology and preserved in 10% neutral buffered formol saline.

head*
larynx*
trachea*
lungs
mediastinal lymph nodes*
jejunum

Tissues marked * were stored.

4.7.8 Tissue processing

10% neutral buffered formol saline was infused into the lungs via the trachea using a syringe. Samples of the lung tissue for electron microscopy were removed after fixation and transferred to a glutaraldehyde solution for post fixation.

The lung and jejunum were processed for subsequent BrdU immunostaining and additional sections of lung were sectioned at 5µ, and stained with haematoxylin and eosin

4.7.9 Microscopic examination

Sections stained with haematoxylin and eosin were examined by light microscopy.

The BrdU labelling index in the terminal bronchioles was evaluated separately from that in the centro-acinar bronchiolar regions. For each area, a sufficient number of regions was assessed to give an approximate total number of cells for each area for each animal of 2000 cells. The number of bronchiolar and alveolar cell that entered DNA synthesis over the labelling period were evaluated as a ratio against the total number of cells.

The glutaraldehyde post-fixed samples of lung tissue were embedded in epoxy resin and semithin sections were prepared. Toluidine blue stained semithin slides were examined and assessed. From selected areas ultrathin sections were prepared and examined by electron microscopy.

5. DATA EVALUATION

All data were evaluated using analysis of variance and/or covariance using the GLM Procedure in SAS (1989) package. Details of the statistical methods used are given in Appendix G.

6. RESULTS

6.1 Atmosphere analysis (Tables 1 and 2)

6.1.1 Particulate concentrations

The mean concentrations [± standard deviations (SD)] were as follows.

Group	Target concentration (mg/m^3)	Measured particulate concentration (mg/m^3) Mean ± SD
2	10	9.9±1.9
3	30	27±5.7
4	100	90.1±8.1

6.1.2 Analysed atmospheric concentrations

The analysed concentrations are given in Table 1. These indicate lower than expected analysed concentrations which are considered attributable to derivatisation problems. The gravimetric concentrations (Section 6.1.1) are considered to represent the true concentrations and hence will be used to subsequently describe the test groups.

6.1.3 Aerodynamic particle size distribution

The aerodynamic particle size distribution of the total particulate were determined to be as follows:

Group	Median size (MMAD) (μm)	Geometric standard deviation
2	1.09	1.48

3	1.25	1.54
4	0.96	1.6

Individual values are given in Table 2.

6.2 Clinical observations (Table 3-4)

6.2.1 Mortality

There were no deaths.

6.2.2 Observations during exposure

The following changes were noted and tended to increase in incidence as the exposure progressed.

Group 1 (control): wet fur, stains around the nose

Group 2 (9.9mg/m^3): wet fur, stains around the nose

Group 3 (27mg/m^3): wet fur, stains around the nose

Group 4 (90.1mg/m^3): wet fur, stains around the nose, breathing depth increased, auditory hypoaesthesia (Table 3).

6.2.3 Observations immediately after exposure and during the maintenance period

The following changes were noted.

Group 1 (control) on days 1-2 hunched, piloerection, stains around the nose and wet fur.

Group 2 (9.9mg/m^3) on days 1-5 hunched, piloerection, stains around the nose, wet fur, chromodacryorrhea, and abnormal respiratory noise.

Group 3 (27mg/m^3) on days 1-8 hunched, piloerection, stains around the nose, wet fur, chromodacryorrhea, lachrymation, sides pinched in, abnormal respiratory noise, breathing depth increased, breathing rate reduced/increased, bulging eyes, mucus secretion from the nose and ungroomed appearance.

Group 4 (90.1mg/m^3) on days 1-7 hunched, piloerection, wet fur, chromodacryorrhea, lachrymation, abnormal respiratory noise, breathing depth increased, breathing rate reduced and/or increased, stains around the nose, mucus secretion from the nose, ungroomed appearance, sides pinched in and activity decreased.

The severity and incidence of the clinical changes decreased with time (Tables 4 and 5).

6.3 Bodyweights (Table 5 Figure 1)

At an exposure concentration of 90.1mg/m^3 there was a marked bodyweight loss on day 2 with most animals remaining below their initial weight on day 4 and 1 animal remaining below its initial weight on day 8. After adjustment for initial weight, bodyweights were approximately 12% below controls on day 2, 7% below controls on day 4 and 3-5% below controls on day 8. Bodyweights were comparable to controls after day 8.

At an exposure concentration of 27mg/m^3 there was a marked bodyweight loss on day 2 with some animals remaining below their initial weight on day 4. After adjustment for initial weight, bodyweights were approximately 9% below controls on day 2, 5% below controls on day 4 and 3-4% below controls on day 8. Bodyweights were comparable to controls after day 8.

At an exposure concentration of 9.9mg/m^3 there was a small bodyweight loss seen in most animals on day 2 although bodyweight was only 2% lower after adjustment for initial weights and this is considered too small to be of any toxicological significance. No effects were seen after day 2.

6.4 Investigations *post mortem* (Tables 6-11,Figures 2-Photographic Supplement)

6.4.1 Lung weights

At an exposure concentration of 90.1mg/m^3 there was a clear increase on day 2 (176%), day 4 (50%) and day 11 (19%). No effects were seen on day 31. At an exposure concentration of 27mg/m^3 there was a clear increase on day 2 (118% although female 27 very high) and day 4 (21%). Still slightly increased on day 11 (9%). No effects were seen on day 31. No effects were seen at any timepoint at an exposure concentration of 9.9mg/m^3 (Table 6 Figure 2).

6.4.2 Cytological examination of lung lavage

On day two there was a slight increase in overall cell count at all exposure concentrations. This was due to a rapid dose related increase in polymorphonuclear leucocytes and lymphocytes. There was a slight dose-related reduction in alveolar macrophages and no effect on eosinophils.

On day four there was a clear dose-related increase in overall cell count at all exposure concentrations. This was due to a marked dose related increase in polymorphonuclear leucocytes and alveolar macrophages. Lymphocytes were still increased but to a lesser extent, particularly at the higher exposure concentrations. There was no effect on eosinophils.

On day 11 a few high individual values were still present in the overall cell count at all exposure concentrations but there were no clear effects on any individual cell types.

On day thirty one all cell counts were similar to control values (Table 7, Figure 3).

6.4.3 Lung lavage fluid total protein

At all exposure concentrations there was a marked dose-related increase on day 2 which was still present, but markedly reduced, on day 4 and confined predominantly to animals exposed to 90.1mg/m^3. On days 11 and 31 some values still appeared slightly deviant but the total protein was considered to have returned to normal (Table 7 Figure 4).

6.4.4 Examination of lung lavage fluid (alkaline phosphatase)

On day two alkaline phosphatase activity showed an apparent dose-related increase at all exposure concentrations but had returned to normal at all subsequent time points (Table 7, Figure 5).

6.4.5 Examination of lavage fluid (lactate dehydrogenase)

Lactate dehydrogenase results showed marked variation at and between time points but with an apparent increase in all exposure groups on day 4 (Table 7, Figure 5).

6.4.6 Examination of lavage fluid (N-acetyl glucosaminidase)

At an exposure concentration of 90.1mg/m^3 N-acetyl glucosaminidase activity was slightly increased on day 2. No effects were seen thereafter. At an exposure concentration of

27mg/m^3 activity was slightly higher on day 2 although all individual values were within the control range. No effects were seen thereafter (Table 7).

6.4.7 Lung labelling index (terminal bronchioles)

Overall means show no effect on day 2 and a dose related increase on day 4. There were no effects on days 11 and 31. However on days 2 and 11 there was some individual animals variation (Table 8 Figure 6).

6.4.8 Lung labelling index (centro-acinar alveoli)

Overall means show no effect on day 2 and a dose related increase on day 4. There were no effects on days 11 and 31. However on days 2 and 11 there was some individual animals variation (Table 8 Figure 6).

6.4.9 Macroscopic pathology

Lungs from rats killed on day 2 showed macroscopic evidence of damage in the form of pale and raised areas diffusely present throughout the lobes. These findings were present at similar incidences at all exposure levels. None of the other tissues showed any changes consistent with a compound-induced effect at any of the time points and no macroscopic changes were observed in the lungs after day 2 (Table 9).

6.4.10 Microscopic pathology

Histopathology.

Explanation of histopathology terms used in the report.

In the context of this report the term pneumonitis has been used to describe the low level of inflammatory cells, mostly neutrophils, present both in the interstitium and air spaces. The term "alveolar epithelialization" is used in the current report to describe a number of microscopic changes which occur predominantly at the site of the alveolar duct region but may also be seen within the alveoli themselves. The term "airway proteinaceous effusion" is used to describe both the "fibrinous" deposits seen within the lung and the proteinaceous fluid seen in the alveoli of some animals during the early time points of the experiment. The higher the severity grading the greater is the involvement of the fluid in the alveoli while at slight to minimal severities only the more "fibrinous" deposits are present.

At day 2 a number of changes were observed in the alveoli which were dose-related in terms of both incidence and severity. A prominent feature that was present in all animals at all dose levels was a macrophage accumulation within the airways although a pneumonitis was seen with a minimal intensity in the 9.9mg/m^3 exposure group through to moderate pneumonitis in 2/5 rats in the 90.1mg/m^3 exposure group. A proteinaceous effusion, present in the airways, was also expressed in a dose-dependent manner with the greatest degree of effect being seen in the 90.1mg/m^3 exposure group. A cell exudate was present in the bronchiolar lumen of rats given all exposure levels of polymeric MDI and increased in severity with increasing dose.

At day 4 alveolar macrophage accumulation was still present at all exposure levels but was less severe than that seen at day 2. Pneumonitis was only present at the top exposure level although alveolar epithelialization was seen at all exposure levels. Within the bronchioles, the cell exudate observed in rats killed on day 2 was still present in 1/5 rats exposed to 90.1mg/m^3, but in addition a bronchiolar epithelial hypertrophy/hyperplasia was also present at all exposure levels which increased in incidence and severity in a dose dependent manner.

At day 11, alveolar macrophage accumulation was only present in 2/5 rats at the 9.9mg/m^3 exposure level and showed a minimal severity grading. Epithelialization of the alveoli and thickening of the alveolar duct wall was present at the 27 and 90.1mg/m^3 and the 9.9, 27 and 90.1mg/m^3 exposure levels respectively but the generalised airway affects seen at the earlier time points had, to a large extent, resolved.

At day 31 the only effects remaining in the lungs were seen in 1/5 rats given 9.9mg/m^3, where a minimal pneumonitis was present, and at the 90.1mg/m^3 exposure level where 1/5 rats showed epithelialization of the alveolar duct and a further 1/5 rats showed a cell exudate in the bronchiolar lumen.

6.4.11 Cell Replication.

Terminal Bronchioles.

There was no statistically significant change in labelling index (LI) in the epithelium of the terminal bronchioles at any of the exposure levels examined at the day 2 time point. On day 4 all exposure levels showed a statistically significant elevation in LI which increased in a dose-dependent manner. On day 11 the 27 and 90.1mg/m^3 exposure groups showed a statistically

significant increase in LI over that seen in the control group although the group exposed to 9.9mg/m^3 had equivalent levels of LI to those seen in the control group. There was no increased LI at any of the dose levels at the 31 day time point.

Centro-acinar Alveoli.

There were elevations in the LI at all dose levels in the centro-acinar alveoli in rats killed on day 2 although only the 27mg/m^3 group showed a statistically significant increase. At the 4 day time point all exposure levels of Polymeric MDI showed statistically significant elevations in LI which increased in a dose dependent manner. At the 11 day time point the 27mg/m^3 and 90.1mg/m^3 dose group showed increases in LI although only in the 90.1mg/m^3 exposure group did this achieve statistical significance. There was no change in LI at any exposure level in rats killed on day 31.

6.4.12 Electron microscopic pathology

Day 2

Examination of the lungs of rats exposed to polymeric MDI for 6h and killed on day 2 showed no increase in the number of Type II cells. Despite there being no increase in the number of Type II cells, minimal increases were noted in the size of Type II cell lamellar bodies in top dose animals, (90.1mg/m^3). Associated with this increase in lamellar body size were increases in surfactant in the alveolar macrophages and lumen. In the alveolar macrophages, minimal levels of crystalline hypophase were associated with minimal (27 mg/m^3) to slight (90.1 mg/m^3) increases in lamellar type surfactant. The only other findings noted in the alveolar macrophages were minimal amounts of amorphous material in some top dose (90.1 mg/m^3) animals. This possibly represents polymeric MDI.

In the alveoli lumen, dose related changes were noted in the amount of cell debris. Described as a slight (9.9 and 27 mg/m^3) to moderate (90.1 mg/m^3) increase, these changes in the amount of cell debris were accompanied by slight increases in the amount of crystalline hypophase surfactant.

Days 4 .

By day 4, a dose related Type II cell hyperplasia was evident in the lungs of rats exposed to polymeric MDI. Observations made at the electron microscope show this hyperplasia to be minimal at 9.9 mg/m^3, rising to a moderate increase in top-dose animals, (90.1 mg/m^3). In addition to this Type II cell hyperplasia, Table 11 shows dose-related changes in the lamellar bodies found in Type II cells; the slight increase in lamellar body numbers being associated with moderate increases in their size, (90.1 mg/m^3).

Dose-related changes were also evident in surfactant levels in the alveoli. The results show slight to marked increases in lamellar surfactant, and minimal to slight increases in crystalline hypophase, in the macrophages of animals exposed to 9.9 and 90.1 mg/m^3 respectively. The only other effects noted were minimal amounts of amorphous material in the alveolar macrophages (27 and 90.1 mg/m^3).

As with the first time point, dose related increases in crystalline hypophase were associated with moderate levels of cell debris in the alveolar lumen of top dose animals, (90.1 mg/m^3).

Day 11

By day 11, the hyperplasia noted at day 4 had reduced to a minimal increase in Type II cells in animals exposed to 9.9 and 90.1 mg/m^3, and was not apparent at 27 mg/m^3.

With only a slight increase in lamellar body size being detected in top-dose (90.1 mg/m^3) animals, the results show that lamellar body numbers had returned to those of controls by day 11. Similarly, although dose related increases in lamellar surfactant persisted, the amount of crystalline surfactant seen in the alveolar macrophages was reduced, (described as slight as opposed to marked in animals exposed to 90.1 mg/m^3, days 11 and 4 respectively).

The only other treatment related findings noted were the presence of minimal amounts of cell debris in the alveolar lumen at all dose levels. The minimal amounts of crystalline hypophase seen in animals exposed to 27 mg/m^3 were considered not to be compound-related since they were similar to those seen in controls at day 4.

No amorphous material was detected in the alveolar macrophages at day 11.

Day 31

Analysis of the lung samples at day 31 showed no effect on the Type II cells. Similarly with macrophage surfactant levels equivalent to those seen in controls day 4, and identical levels of crystalline hypophase in the lumen of control and top dose animals, no compound-related effects could be detected in animals sacrificed day 31.

Similarly, no amorphous material was noted in the alveolar macrophages at day 31 (Table 11).

7. DISCUSSION

The purpose of this study was to investigate the 6-hour acute inhalation toxicity of polymeric MDI and this was accomplished successfully. The health of the rats was satisfactory and there was no evidence of disease or infection which might have compromised the interpretation of the findings.

The test atmospheres generated at concentrations of 9.9, 27 or 90.1mg/m^3 were acceptable with regard to their general stability and physical characteristics.

Clinical changes such as breathing irregularities, mucus secretion and lachrymation etc. indicative of irritancy were present in all animals exposed to polymeric MDI and showed a dose-related increase in incidence and severity. Clinical findings of wet fur, stains around the nose, hunched appearance and piloerection seen during exposure and in the early post-exposure period are generally associated with restraint and considered to be of no toxicological significance. Bodyweights of animals exposed to 90.1 or 27mg/m^3 were reduced by approximately 12 and 9% respectively, compared to their initial weights on day 2 of the study. The bodyweights of all animals were comparable to controls by the end of the study. Bodyweights of animals exposed to 9.9mg/m^3 were not significantly different from their initial values on day 2 of the study.

There was a dose-related increase in lung weight in all animals exposed to 27 or 90.1mg/m^3 polymeric MDI. The duration of the effect increased with dose but all animals had recovered by day 31.

Examination of lung lavage fluid showed effects, often in a dose-response relationship, in the majority of parameters measured. Total cell counts increased slightly on day 2 and this was

attributable mainly to increases in polymorphonuclear leucocytes. By day 4 total counts were increased further at the two highest concentrations and this was attributable at this time point to increased numbers of macrophages and polymorphonuclear leucocytes together with an increase in lymphocyte counts. The polymorphonuclear leukocyte counts imply that there is a rapid response by this cell type to exposure but that by day 4 there is already evidence of recovery. Total protein, alkaline phosphatase and N-acetyl glucosaminidase showed rapid increases following exposure, with maximal effect at day 2 and a virtual return to normal by day 4. The results for lactate dehydrogenase showed marked variation such that full interpretation was difficult but it is possible that this parameter showed greater effects at day 4.

It is worth noting the apparent discrepancy between the number of alveolar macrophages lavaged from the lungs on days 2 and 4 and the respective histopathological descriptions. On day 2 lavage numbers of macrophages were lower than control at all concentrations whilst at day 4 they were significantly greater. This contrasts with the histological observation of increased macrophage accumulation at day 2 with a reduction in the severity at day 4. While there is no clear explanation for this, it is possible that the freshly accumulated and presumably active macrophages are less amenable to lavage than other cell types. Alternatively, the high concentrations of N-acetyl glucosaminidase measured in the lavage supernatant is often an indicator of damage to macrophages. Hence, washing out of the lung and centrifugation of such cells may cause further damage to the extent that the cells disintegrate and are observed only as debris in the differential counts. Both hypotheses are plausible and the fact that the total cell counts in the lavage fluid increase over control at these two times point to a macrophage specific phenomenon rather than procedural differences.

BrdU labelling showed similar patterns for both the terminal bronchioles and centro-acinar alveoli. Mean values indicate a delay in response, no effects of significance being apparent at day 2 but a clear dose related increase at day 4. Evaluation of individual animal values showed that some were beginning to respond by day 2 but most activity was seen at day 4.

Microscopic analysis of the lungs showed changes consistent with exposure to a highly irritant aerosol but that after a single exposure recovery was apparent. Electron microscopy confirmed these general findings but showed together with Type II cell hyperplasia there was

an effect on lung surfactant, with increases being apparent in Type II cells, in the alveolar lumen and the quantities phagacytosed by alveolar macrophages.

All parameters had returned to normal by the end of the study.

8. CONCLUSION

Nose-only exposure for 6 hours to polymeric MDI at concentrations of 9.9, 27 or 90.1mg/m^3 resulted in effects at all concentrations. These included clinical changes, increased excretion of surfactant, increases in cellular exudates and related enzyme activities, a Type II cell hyperplasia and increases in the numbers and size of lamellar bodies within the Type II cells. These changes showed a dose-related increase in severity and incidence. There was an adverse effect on bodyweights in animals at 27 and 90.1mg/m^3 but at 9.9mg/m^3 bodyweights were not affected.

All animals showed full recovery by day 31 of the study.

9. REFERENCES

Freeman M F and Tukey J W (1950). Transformations related to the angular and the square root. Annals of Maths Stats 21, 607.

SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2, Cary, NC: SAS Institute INC., 1989.

Shirley E (1996). A literature review of statistical methods for the analysis of general toxicology data. In Statistics in Toxicology (ed. B J T Morgan). Oxford University Press, Oxford.

GLOSSARY FOR FIGURES 1-6

Key:

The nominal concentrations of 10, 30 or 100mg/m^3 quoted on the Figures equate to the achieved concentrations of 9.9, 27 or 90.1mg/m^3 respectively.

Figures 3-6

Bars represent mean +95% confidence limit.

FIGURE 1 - GROUP MEAN BODYWEIGHT VERSUS TIME
DAY 2 KILL

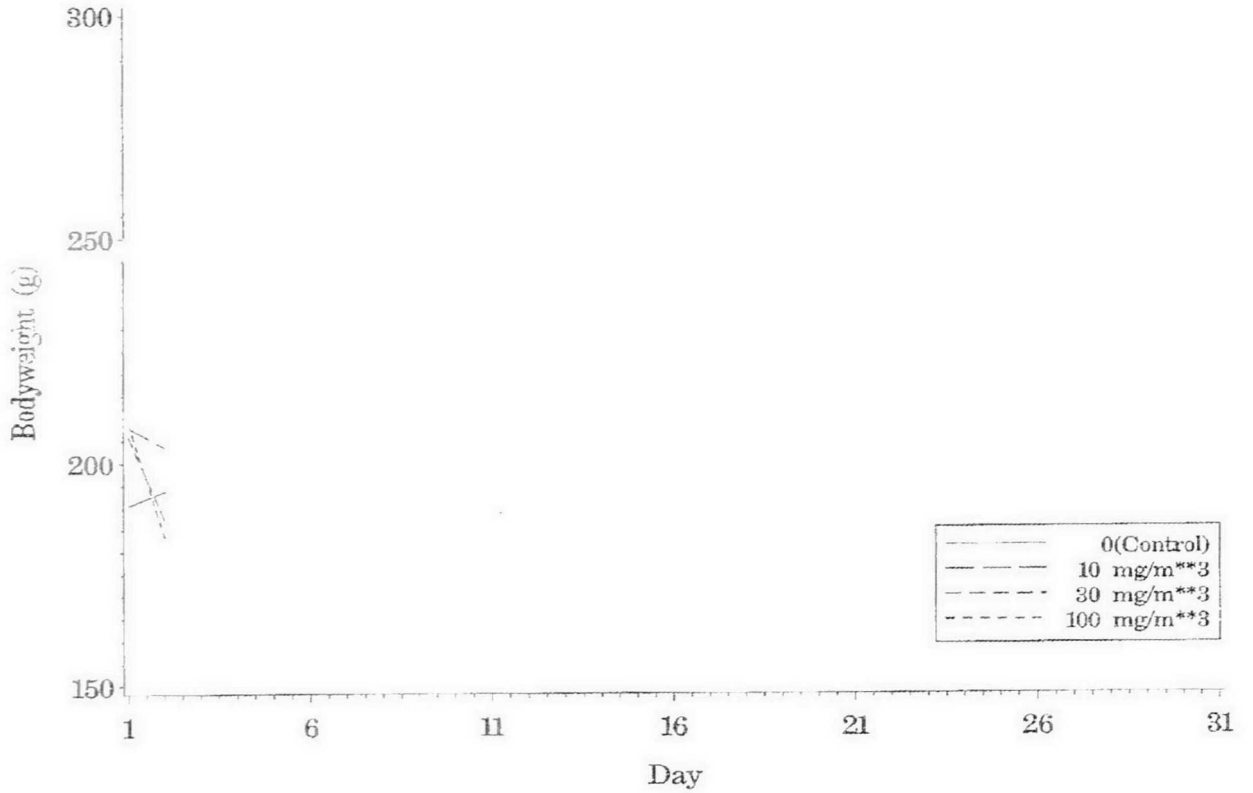


FIGURE 1 - GROUP MEAN BODYWEIGHT VERSUS TIME
DAY 4 KILL

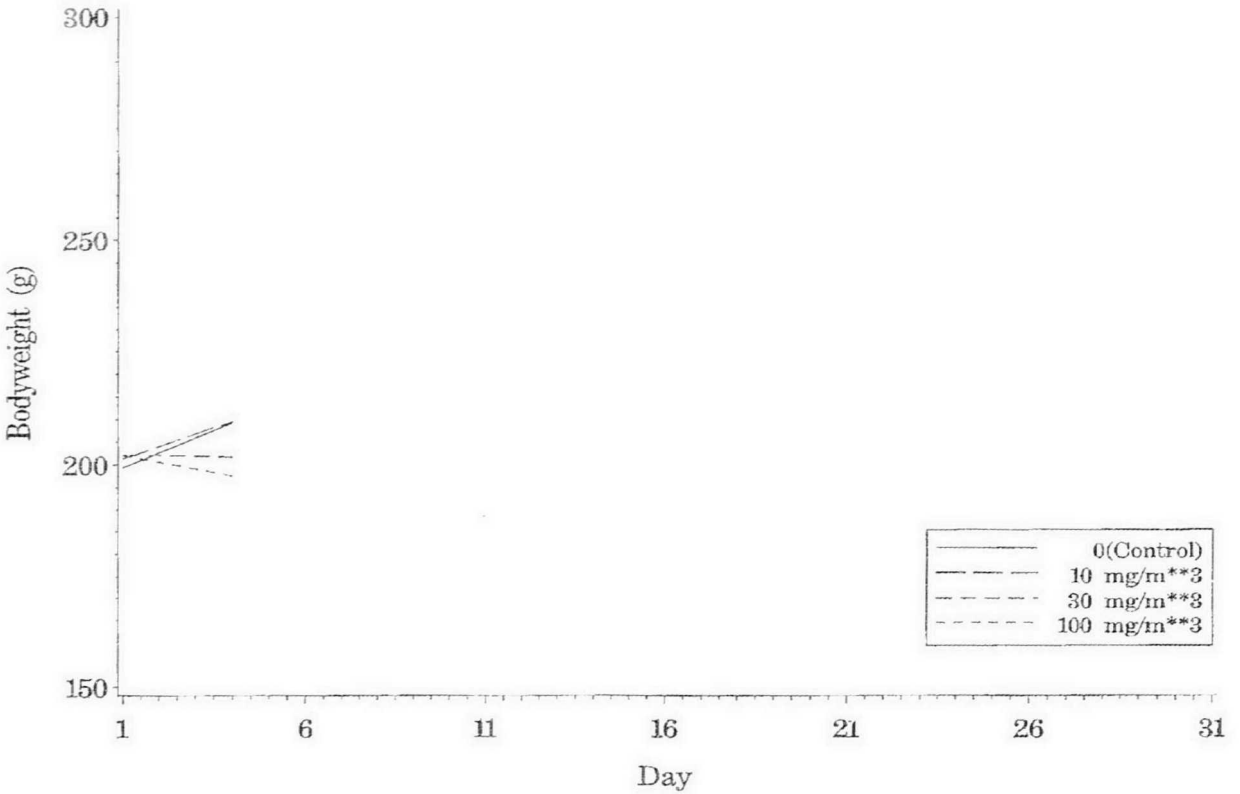


FIGURE 1 - GROUP MEAN BODYWEIGHT VERSUS TIME
DAY 11 KILL

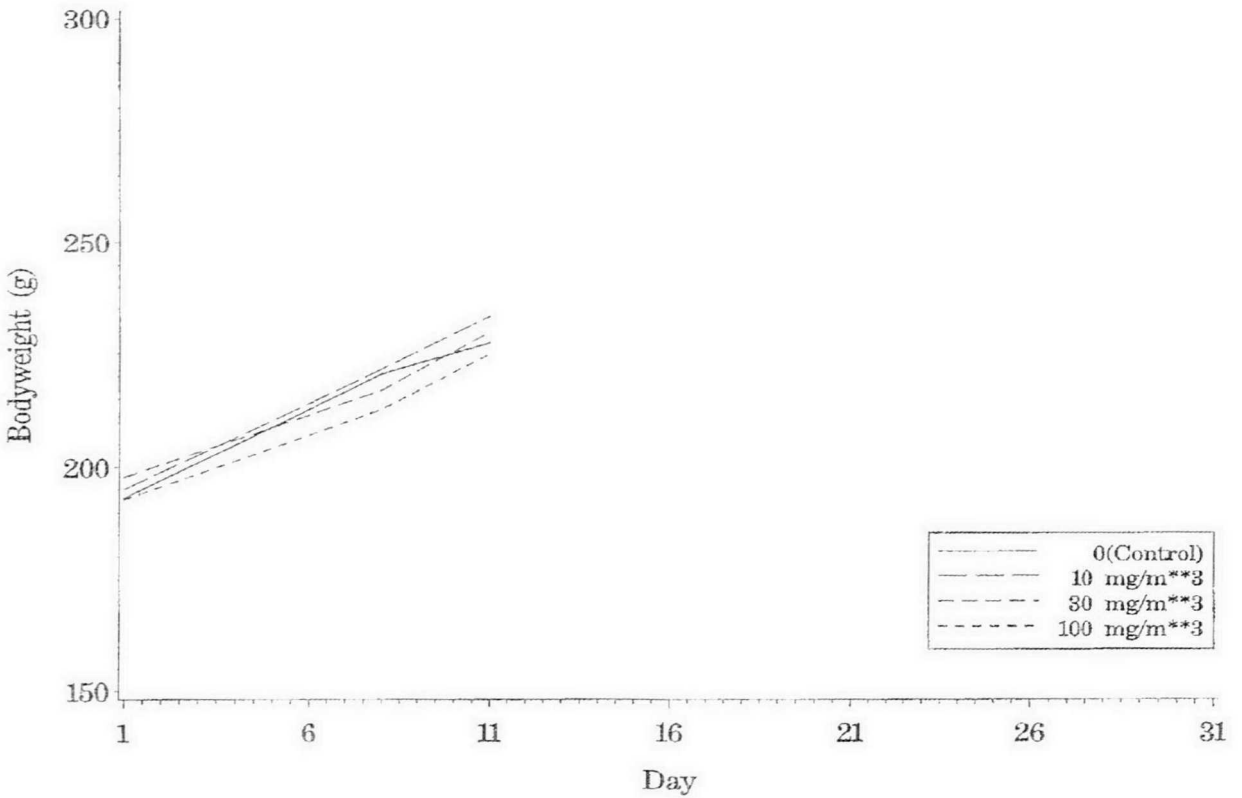


FIGURE 1 - GROUP MEAN BODYWEIGHT VERSUS TIME
DAY 31 KILL

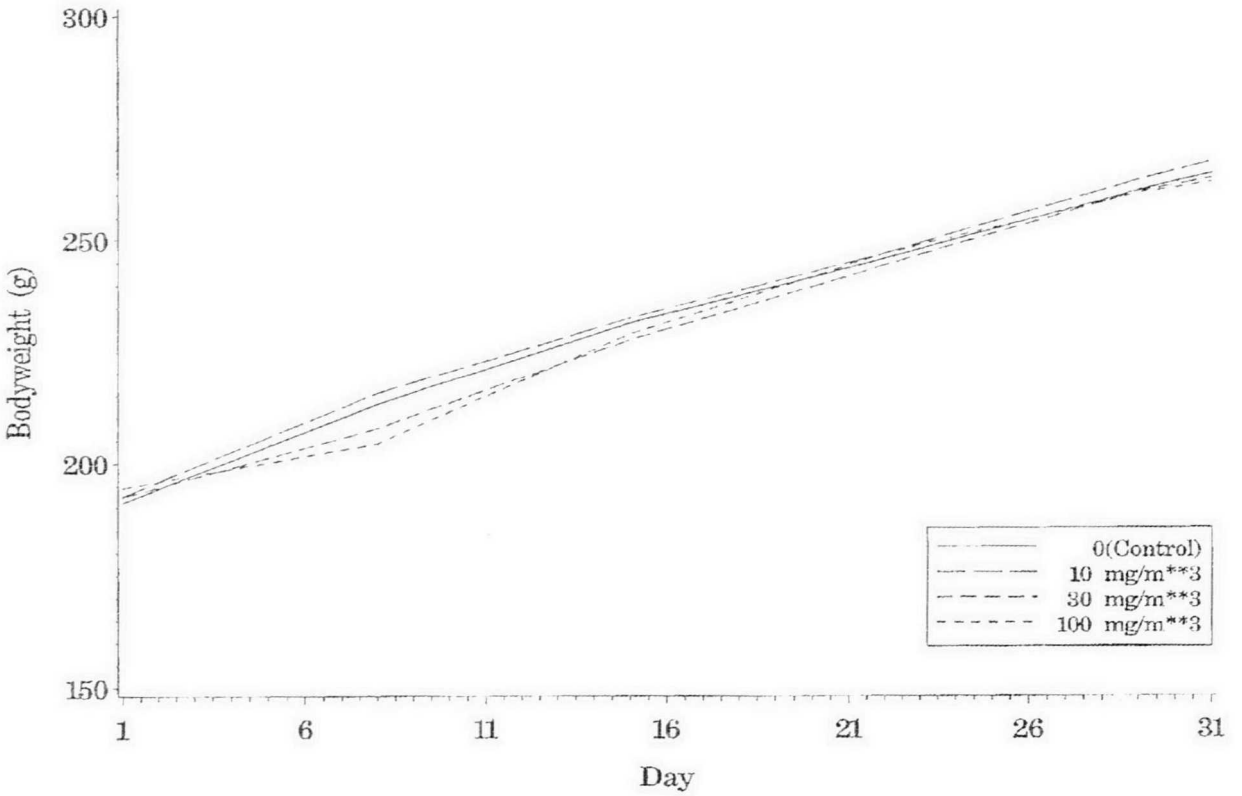


FIGURE 2 - GROUP MEAN LUNG WEIGHT (g)

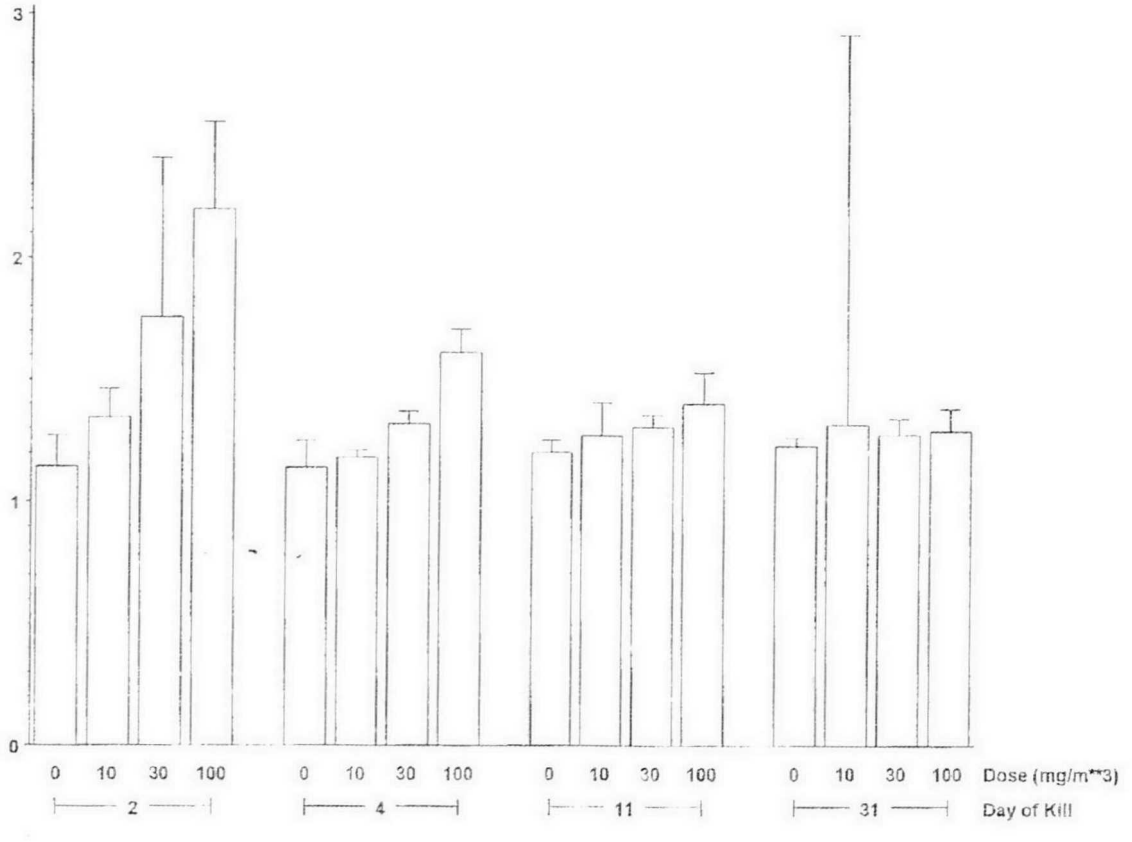


FIGURE 3 - LUNG LAVAGE FLUID
GROUP MEAN TOTAL CELL COUNT (x10**6/ml)

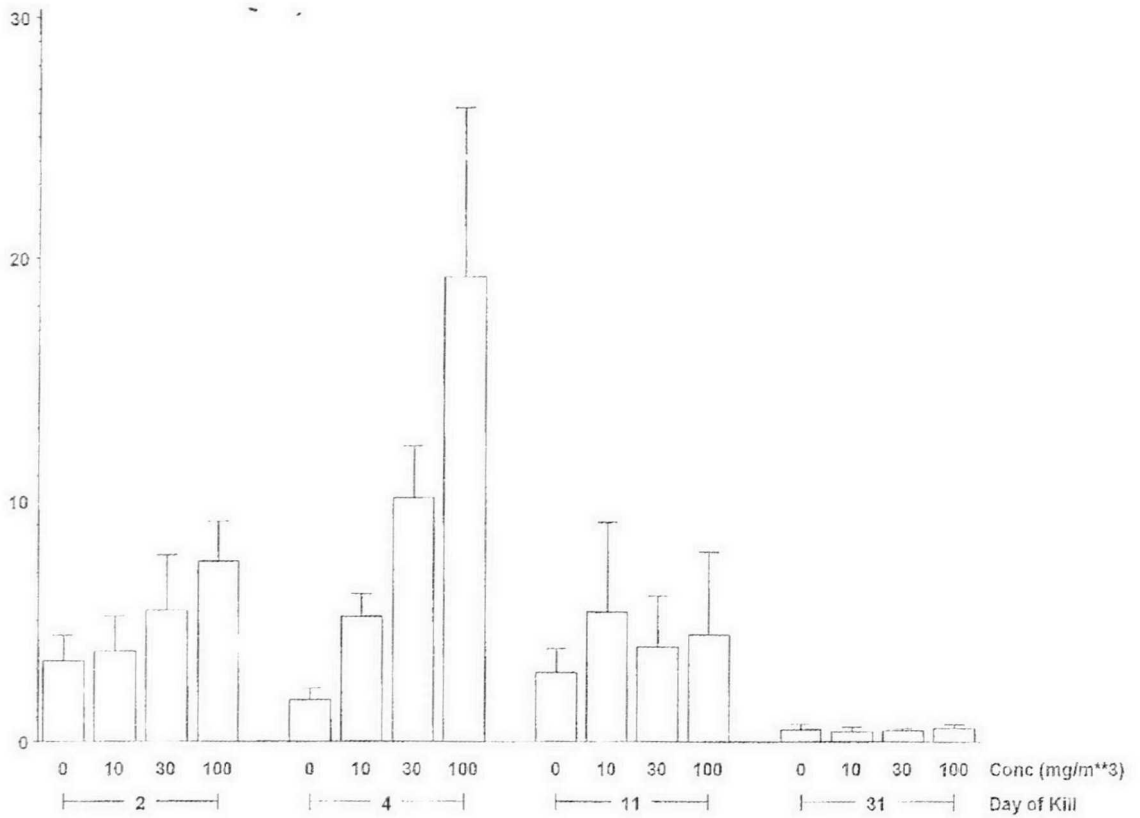


FIGURE 3 - LUNG LAVAGE FLUID
GROUP MEAN ALVEOLAR MACROPHAGE COUNT (x10**6/ml)

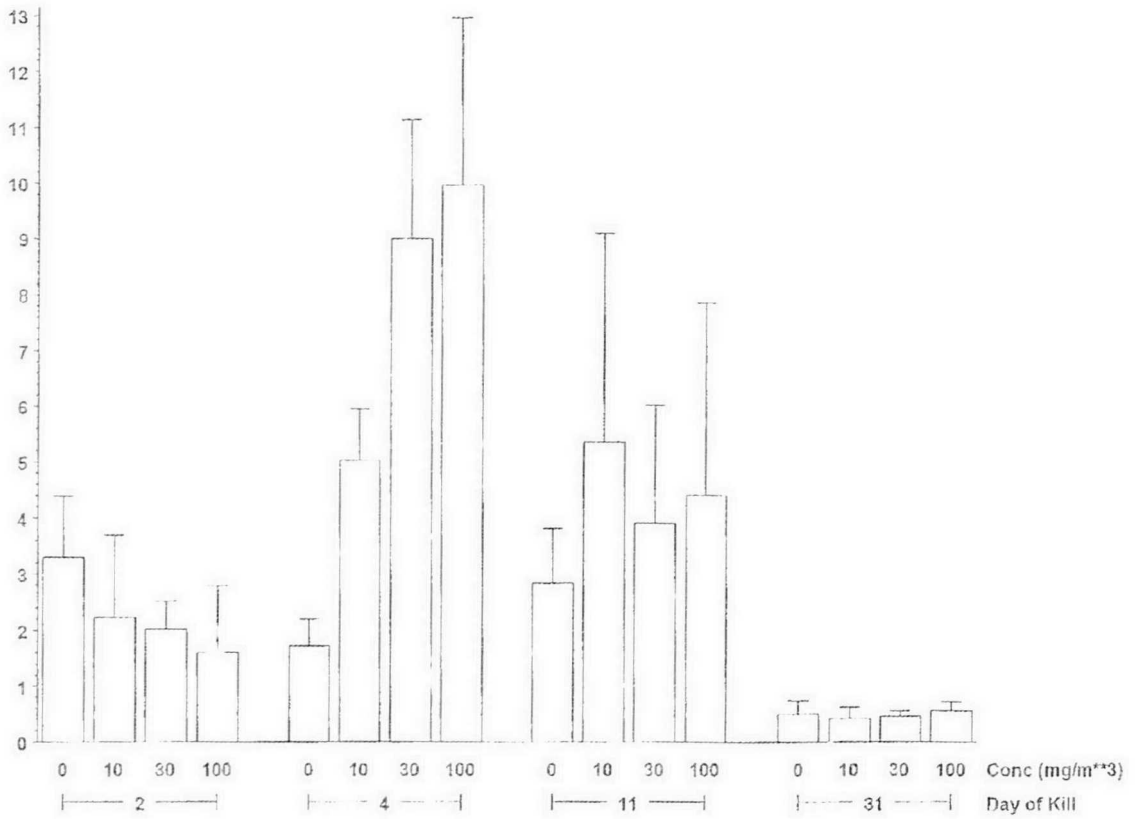


FIGURE 3 - LUNG LAVAGE FLUID
GROUP MEAN POLYMORPHONUCLEAR LEUCOCYTE CELL C[illegible]T (x10**6/ml)

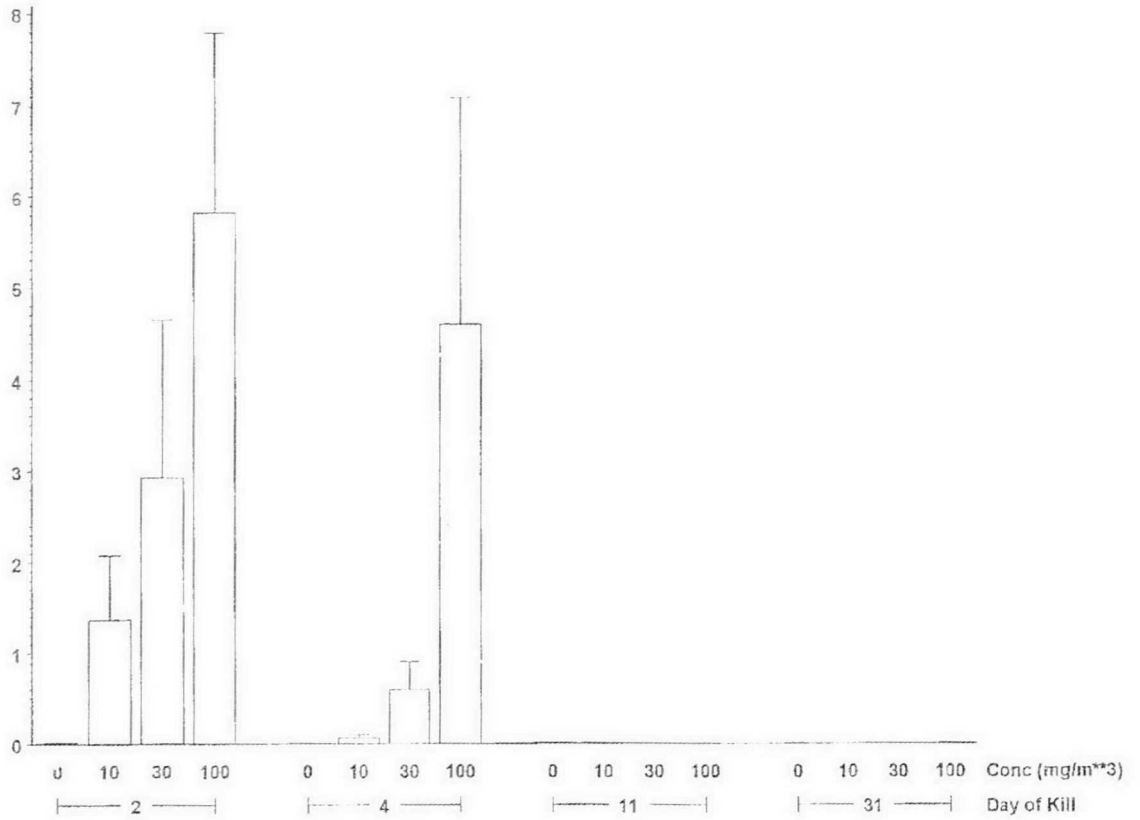


FIGURE 3 - LUNG LAVAGE FLUID
GROUP MEAN LYMPHOCYTE/OTHER COUNT (x10**6/ml)

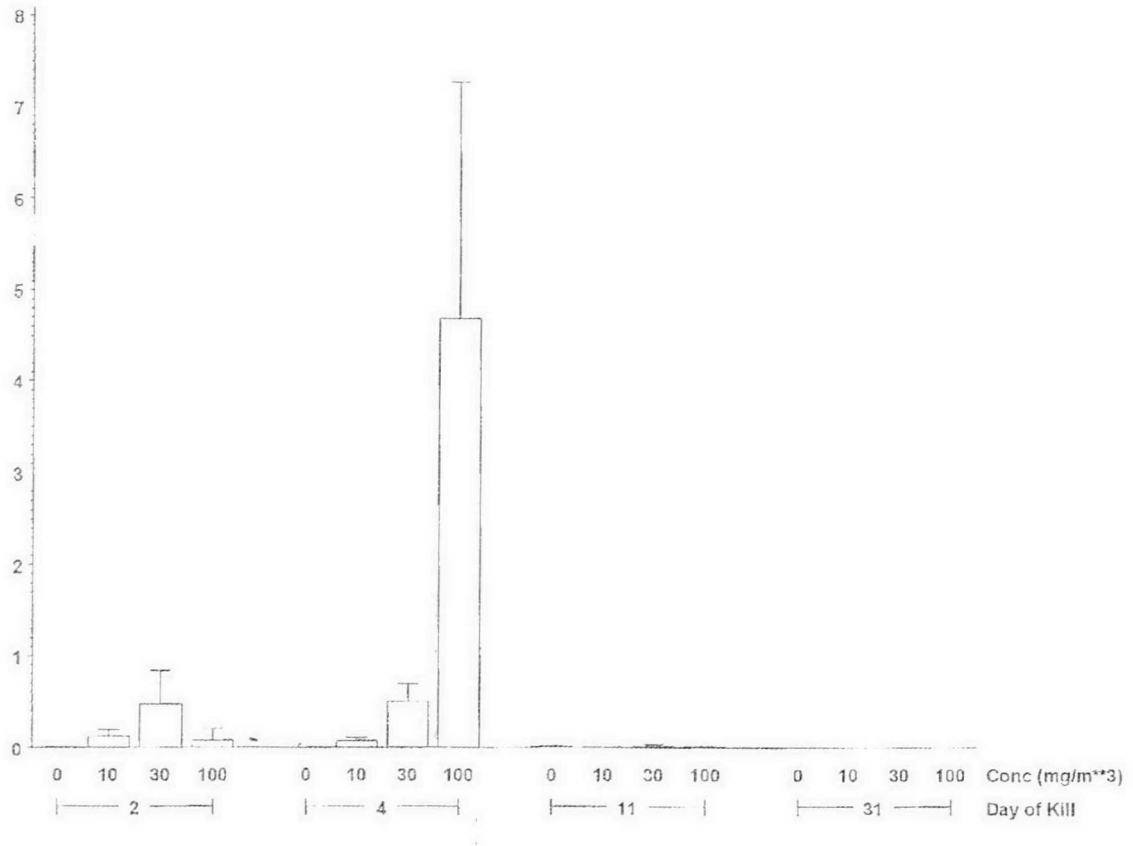


FIGURE 4 - LUNG LAVAGE FLUID
GROUP MEAN TOTAL PROTEIN (mg/dl)

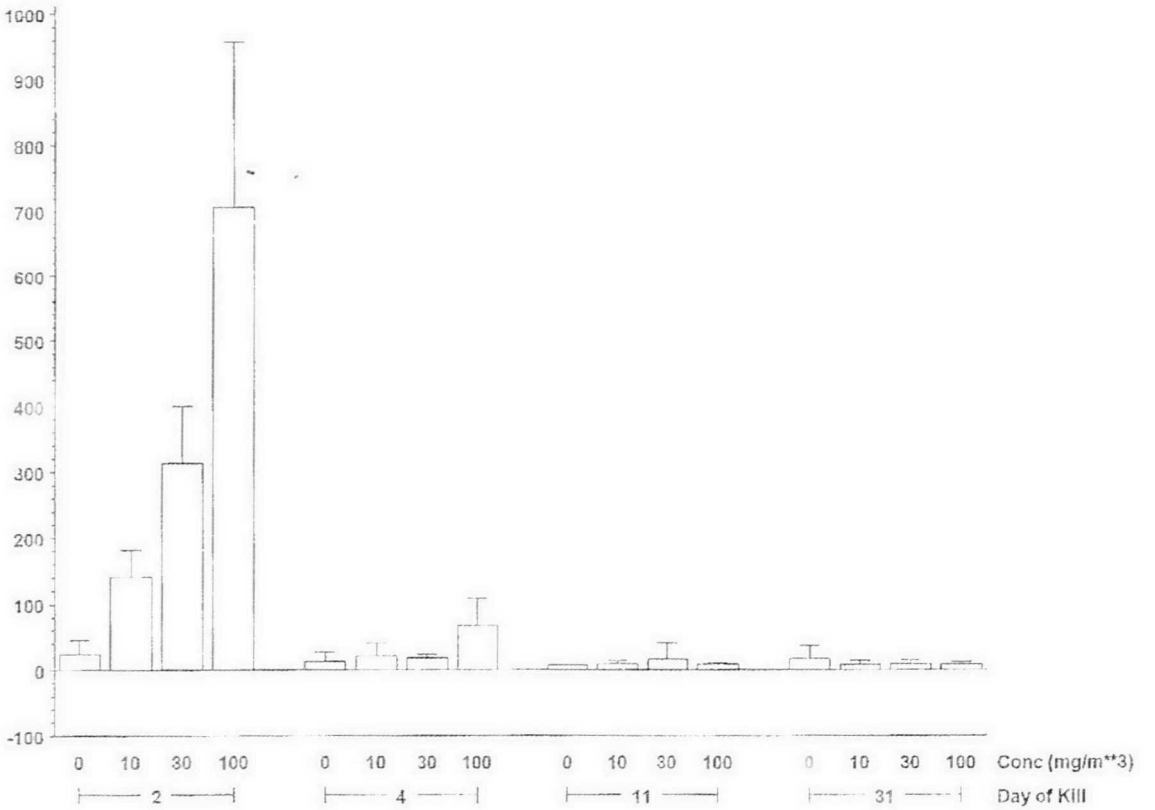


FIGURE 5 - LUNG LAVAGE FLUID
GROUP MEAN LACTATE DEHYDROGENASE (IU/l)

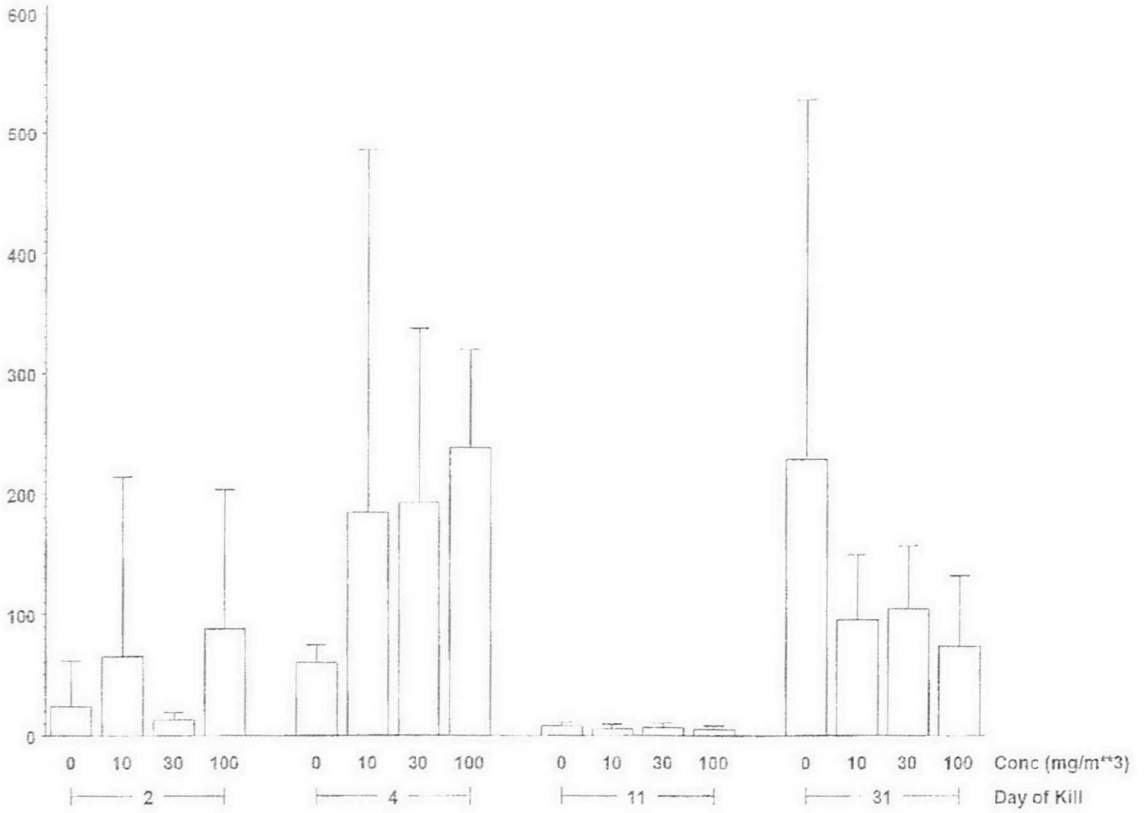


FIGURE 5 - LUNG LAVAGE FLUID
GROUP MEAN ALKALINE PHOSPHATASE (IU/l)

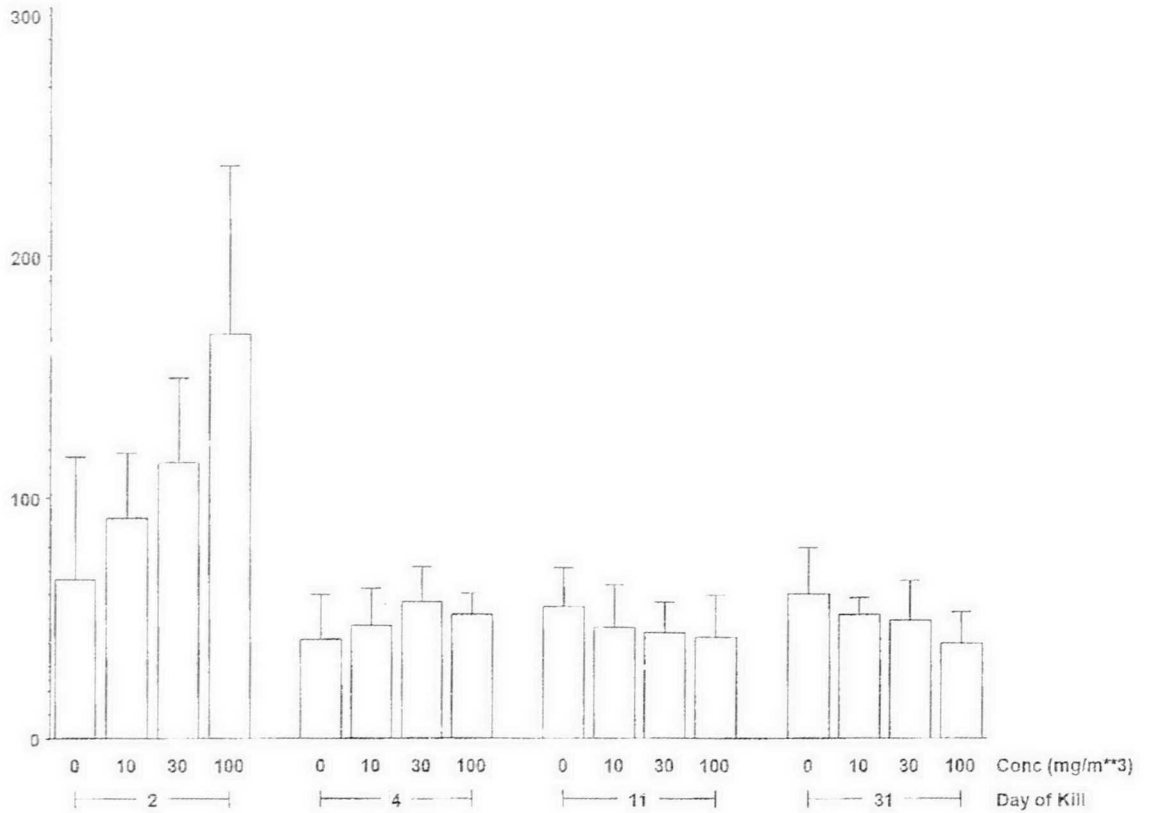


FIGURE 6 - BRDU LABELLING INDICES
GROUP MEAN TERMINAL BRONCHIOLES (%)

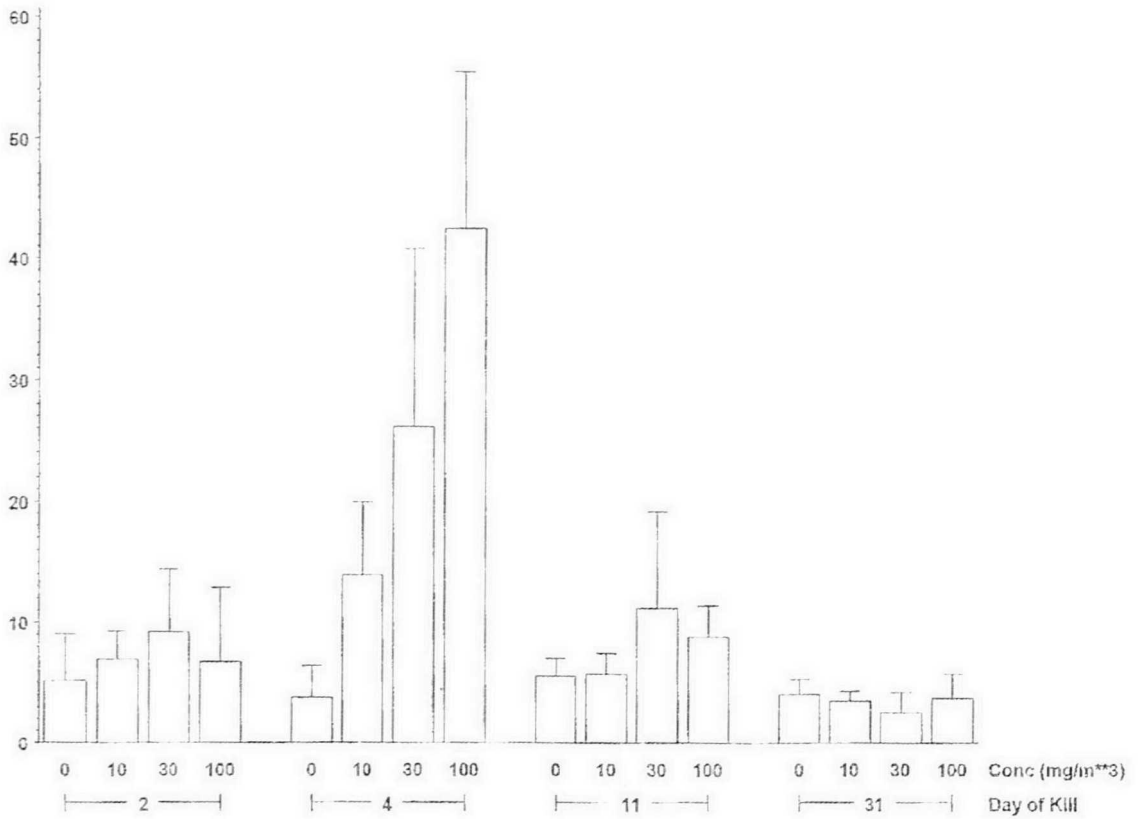


FIGURE 6 - BRDU LABELLING INDICES
GROUP MEAN CENTRO-ACINAR ALVEOLI (%)

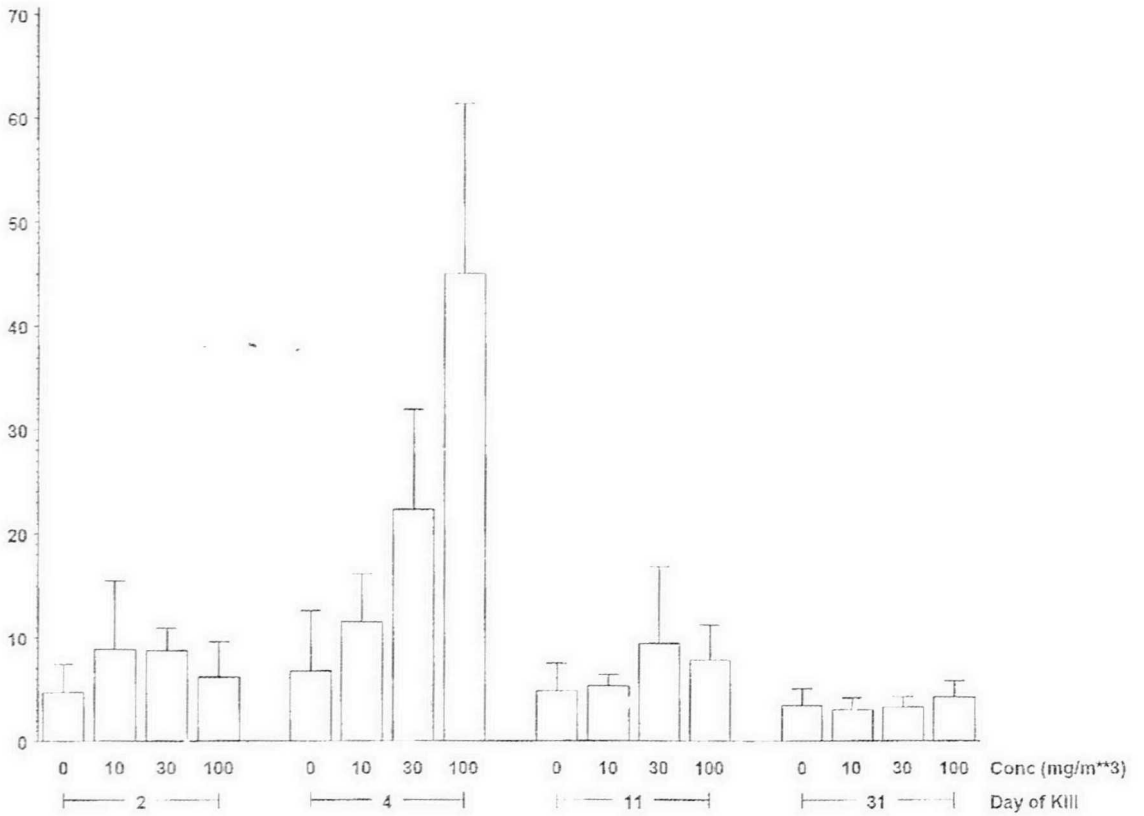


TABLE 1 - ATMOSPHERIC CONCENTRATIONS GROUP 2

Date	Time into exposure (hrs:mins)	Total particulate concentration (ug/l)	Analysed Isocyanate concentration (ug/l)	% Total particulate
17-Nov-97	04:30	10.6	0.46	4.3
	05:47	13.1	0.69	5.3
25-Nov-97	04:37	9.4	5.3	56.4
	05:18	9.9	6.2	62.6
02-Dec-97	03:19	8.5	4.5	52.9
	04:50	7.6	3.8	50.0
	Mean	9.9	3.5	38.6
	Standard deviation	1.9	2.4	26.5

TABLE 1 - ATMOSPHERIC CONCENTRATIONS GROUP 3

Date	Time into exposure (hrs:mins)	Total particulate concentration (ug/l)	Analysed Isocyanate concentration (ug^{n})	% Total particulate
17-Nov-97	05:03	34.7	0.60	1.7
	06:20	31.0	0.72	2.3
25-Nov-97	01:29	22.6	13.0	57.5
	05:31	21.0	9.0	42.9
02-Dec-97	05:05	22.2	11.4	51.4
	06:16	30.4	19.0	62.5
	Mean	27.0	9.0	36.4
	Standard deviation	5.7	7.2	27.4

TABLE 1 - ATMOSPHERIC CONCENTRATIONS GROUP 4

Date	Time into exposure (hrs:mins)	Total particulate concentration (ug/l)	Analysed Isocyanate concentration (ug/l)	% Total particulate
17-Nov-97	02:03	95.8	2.48	2.6
	06:32	94.3	2.80	3.0
25-Nov-97	04:32	97.1	61.4	63.2
	06:07	90.5	51.2	56.6
02-Dec-97	04:44	87.5	43.6	49.8
	05:15	75.1	30.2	40.2
	Mean	90.1	31.9	35.9
	Standard deviation	8.1	24.9	26.7

TABLE 2 - AERODYNAMIC PARTICLE SIZE DISTRIBUTION

Group 2 9.9mg/m^3

Size range (µm)	Time 1	Time 2	Time 3
	% by weight in range	% by weight in range	% by weight in range
	Gravimetric	Gravimetric	Gravimetric
≥9.8	0.01	0.01	0.009
9.8-6.0	0.12	0.01	0.009
6.0-3.5	0.74	0.6	0.186
3.5-1.55	33.74	43.97	53.571
1.55-0.93	35.21	30.39	26.786
0.93-0.52	23.19	10.61	10.696
≤0.52	6.99	14.42	8.734

Percentages are calculated as follows:

Gravimetric: $\frac{\text{weight trapped at each size range}}{\text{total weight trapped}} \times 100$

Note - Gravimetric is equivalent to particulate in this study.

Time 1 First exposure

Time 2 Second exposure

Time 3 Third exposure

TABLE 2 - AERODYNAMIC PARTICLE SIZE DISTRIBUTION

Group 3 27mg/m^3

Size range (μm)	Time 1	Time 2	Time 3
	% by weight in range	% by weight in range	% by weight in range
	Gravimetric	Gravimetric	Gravimetric
≥9.8	0.26	0.01	0.130
9.8-6.0	0.32	0.01	0.007
6.0-3.5	1.54	1.37	0.391
3.5-1.55	44.59	47.07	19.074
1.55-0.93	31.39	31.73	52.471
0.93-0.52	19.92	16.08	24.022
≤0.52	1.99	3.74	3.906

Percentages are calculated as follows:

Gravimetric: $\frac{\text{weight trapped at each size range}}{\text{total weight trapped}} \times 100$

Note - Gravimetric is equivalent to particulate in this study.

Time 1 First exposure

Time 2 Second exposure

Time 3 Third exposure

TABLE 2 - AERODYNAMIC PARTICLE SIZE DISTRIBUTION

Group 4 90.1mg/m^3

Size range (µm)	Time 1	Time 2	Time 3
	% by weight in range	% by weight in range	% by weight in range
	Gravimetric	Gravimetric	Gravimetric
≥9.8	0.10	0.26	0.005
9.8-6.0	0.00	0.11	0.005
6.0-3.5	1.06	0.16	0.215
3.5-1.55	43.69	5.48	5.182
1.55-0.93	30.41	43.44	33.689
0.93-0.52	19.63	44.76	39.931
≤0.52	5.10	5.79	20.342

Percentages are calculated as follows:

Gravimetric: $\frac{\text{weight trapped at each size range}}{\text{total weight trapped}} \times 100$

Note - Gravimetric is equivalent to particulate in this study.

Time 1 First exposure

Time 2 Second exposure

Time 3 Third exposure

TABLE 3- CLINICAL OBSERVATIONS DURING EXPOSURE

Group 1 (control)

Approximate time into exposure (mins)	Abnormalities
30	Some animals had wet fur.
60	Some animals had wet fur.
90	Some animals had wet fur and some showed stained noses.
120	Some animals had wet fur and some showed stained noses.
150	Some animals had wet fur and some showed stained noses.
180	Some animals had wet fur and some showed stained noses.
210	Some animals had wet fur and some showed stained noses.
240	Some animals had wet fur and some showed stained noses.
270	Some animals had wet fur and some showed stained noses.
300	All animals had wet fur and some showed stained noses..
330	All animals had wet fur and some showed stained noses.

TABLE 3 - CLINICAL OBSERVATIONS DURING EXPOSURE

Group 2 (9.9mg/m^3)

Approximate time into exposure (mins)	Abnormalities
30	Some animals had wet fur.
60	Some animals had wet fur.
90	Some animals had wet fur and some showed stained noses.
120	Some animals had wet fur and some showed stained noses.
150	Some animals had wet fur and some showed stained noses.
180	Some animals had wet fur and some showed stained noses.
210	Some animals had wet fur and some showed stained noses.
240	Some animals had wet fur and some showed stained noses.
270	Some animals had wet fur and some showed stained noses.
300	All animals had wet fur and some showed stained noses.
330	All animals had wet fur and some showed stained noses.

TABLE 3 - CLINICAL OBSERVATIONS DURING EXPOSURE

Group 3 (27mg/m^3)

Approximate time into exposure (mins)	Abnormalities
30	Some animals had wet fur.
60	Some animals had wet fur.
90	Some animals had wet fur and some showed stained noses.
120	Some animals had wet fur and some showed stained noses.
150	Some animals had wet fur and some showed stained noses.
180	Some animals had wet fur and some showed stained noses.
210	Some animals had wet fur and some showed stained noses.
240	Some animals had wet fur and some showed stained noses.
270	All animals had wet fur and some showed stained noses.
300	All animals had wet fur and some showed stained noses.
330	All animals had wet fur and some showed stained noses.

TABLE 3 - CLINICAL OBSERVATIONS DURING EXPOSURE

Group 4 (90.1mg/m^3)

Aproximate time into exposure (mins)	Abnormalities
30	Some animals had wet fur.
60	Some animals had wet fur.
90	Some animals had wet fur and some showed stained noses.
120	Some animals had wet fur and some showed stained noses.
150	Some animals had wet fur and some showed stained noses.
180	Some animals had wet fur and some showed stained noses.
210	Some animals had wet fur and some showed stained noses.
240	Some animals had wet fur and some showed stained noses and some showed increased breathing depth.
270	All animals had wet fur and some showed stained noses and some showed increased breathing depth.
300	All animals had wet fur and some showed stained noses and some showed increased breathing depth.
330	All animals had wet fur and some showed stained noses and some showed increased breathing depth.

GLOSSARY FOR TABLE 4

Key:-

NO. OF OBS	-	Number of observations. This may represent the recording of an observation on more than one occasion during the day for any individual animal.
NO	-	number
NAD	-	no abnormalities detected
S	-	slight
M	-	moderate
E	-	extreme
X	-	noted
N	-	abnormality no longer present
I	-	increased) applies to breathing rate/depth
R	-	reduced)
B	-	both)
L	-	left) applies to eyes
R	-	right)
SUBS	-	substance
INCR/SD	-	increased
DEC/SD	-	decreased
PLAC.	-	placing
RESP.	-	response
RESPIRAT/Y	-	respiratory
CURV'URE	-	curvature
(D)	-	dead
wt	-	weight
wks	-	weeks
SEX: M	-	male
SEX: F	-	female

NB The above abbreviations may appear in this Table.

SEE FREE TEXT - free text comments are displayed in the IAD:-

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

SEX: FEMALES

0 mg/m3	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
HUNCHED																					
SEVERITIES S	40	2																			
TOTAL NO. OF OBS.	40	2																			
KILLED TERMINATION																					
SEVERITIES V		10		10							10										
TOTAL NO. OF OBS.		10		10							10										
PILOERECTION																					
SEVERITIES S	40	35	1																		
TOTAL NO. OF OBS.	40	35	1																		
STAINS AROUND NOSE																					
SEVERITIES X	15																				
TOTAL NO. OF OBS.	15																				
WET FUR																					
SEVERITIES M	15																				
S	25																				
TOTAL NO. OF OBS.	40																				

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

0
mg/m3

SEX: FEMALES

	DAY NUMBERS									
	22	23	24	25	26	27	28	29	30	31
KILLED TERMINATION										
SEVERITIES X										10
TOTAL NO. OF OBS.										10

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

9.9 mg/m3 — SEX: FEMALES

		DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
CHROMODACRYORRHEA																						
SEVERITIES	B	1																				
	L			1																		
TOTAL NO. OF OBS.		1		1																		
HUNCHED																						
SEVERITIES	S	40	6																			
	X			1																		
TOTAL NO. OF OBS.		40	6	1																		
KILLED TERMINATION																						
SEVERITIES	X		10		10							10										
TOTAL NO. OF OBS.			10		10							10										
PILOERECTION																						
SEVERITIES	S	40	35	1	3	1																
	X			8																		
TOTAL NO. OF OBS.		40	35	9	3	1																
ABNORMAL RESPIRAT/Y NOISE																						
SEVERITIES	X	7	4	2	2	1																
TOTAL NO. OF OBS.		7	4	2	2	1																
STAINS AROUND NOSE																						
SEVERITIES	X	22																				
TOTAL NO. OF OBS.		22																				
WET FUR																						
SEVERITIES	M	13																				
	S	27																				
TOTAL NO. OF OBS.		40																				

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

9.9 mg/m3 SEX: FEMALES

	DAY NUMBERS 22	23	24	25	26	27	2.	29	30	31
KILLED TERMINATION										
SEVERITIES X										10
TOTAL NO. OF OBS.										10

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

27.0 mg/m3 — SEX: FEMALES

		DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
BREATHING DEPTH																						
SEVERITIES	I	5	22	7	5	1																
TOTAL NO. OF OBS.		5	22	7	5	1																
BREATHING RATE																						
SEVERITIES	I			5		1																
	R		2	1	2																	
TOTAL NO. OF OBS.			2	6	2	1																
CHROMODACRYORRHEA																						
SEVERITIES	B		1																			
	L	1	2																			
	R	2	1	1																		
TOTAL NO. OF OBS.		3	4	1																		
EYE BULGING																						
SEVERITIES	B						1															
	L				1	1		1														
	R			1																		
TOTAL NO. OF OBS.				1	1	1	1	1														
HUNCHED																						
SEVERITIES	S	40	35	4	4																	
	X			11																		
TOTAL NO. OF OBS.		40	35	15	4																	
KILLED TERMINATION																						
SEVERITIES	X		10		10							10										
TOTAL NO. OF OBS.			10		10							10										
LACHRYMATION																						
SEVERITIES	B	6																				
TOTAL NO. OF OBS.		6																				

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

27.0 mg/m3 — SEX: FEMALES

	DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
PILOERECTION																					
SEVERITIES M	1	3																			
S	39	33	10	17	9	5															
X			14			1															
TOTAL NO. OF OBS.	40	36	24	17	9	6															
ABNORMAL RESPIRAT/Y NOISE																					
SEVERITIES X	29	37	14	12	6	2	1	1													
TOTAL NO. OF OBS.	29	37	14	12	6	2	1	1													
MUCUS SECRETION FROM NOSE																					
SEVERITIES X	6	10																			
TOTAL NO. OF OBS.	6	10																			
SIDES PINCHED IN																					
SEVERITIES S		5																			
TOTAL NO. OF OBS.		5																			
STAINS AROUND NOSE																					
SEVERITIES X	36	4	1																		
TOTAL NO. OF OBS.	36	4	1																		
UNGROOMED																					
SEVERITIES X		10																			
TOTAL NO. OF OBS.		10																			
WET FUR																					
SEVERITIES M	17																				
S	23																				
TOTAL NO. OF OBS.	40																				

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

27 0 mg/m3 — SEX: FEMALES

	DAY NUMBERS 22	23	24	25	26	27	28	29	30	31
KILLED TERMINATION										
SEVERITIES X										10
TOTAL NO. OF OBS.										10

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

90.1 mg/m3		SEX: FEMALES																			
		DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
ACTIVITY DECREASED																						
SEVERITIES	M		1	1																		
	S	40	39	9	1																	
TOTAL NO. OF OBS.		40	40	10	1																	
BREATHING DEPTH																						
SEVERITIES	I	40	40	25	22	11	2															
TOTAL NO. OF OBS.		40	40	25	22	11	2															
BREATHING RATE																						
SEVERITIES	I			19			1	1														
	R	40	40	5	13	1																
TOTAL NO. OF OBS.		40	40	24	13	1	1	1														
CHROMODACRYORRHEA																						
SEVERITIES	B		4	1																		
	L	1	4	2																		
	R		1	2																		
TOTAL NO. OF OBS.		1	9	5																		
HUNCHED																						
SEVERITIES	M		7																			
	S	40	28	12	12	2																
	X			15																		
TOTAL NO. OF OBS.		40	35	27	12	2																
KILLED TERMINATION																						
SEVERITIES	X		10		10							10										
TOTAL NO. OF OBS.			10		10							10										
LACHRYMATION																						
SEVERITIES	B	11	2																			
TOTAL NO. OF OBS.		11	2																			

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EYPOSURE

90.1 mg/m3 — SEX: FEMALES

		DAY NUMBERS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
PILOERECTION																						
SEVERITIES	M	33	27																			
	S	7	11	10	30	18	9	2														
	X			20			3															
TOTAL NO. OF OBS.		40	38	30	30	18	12	2														
ABNORMAL RESPIRAT/Y NOISE																						
SEVERITIES	X	40	40	26	26	15	6															
TOTAL NO. OF OBS.		40	40	26	26	15	6															
RIGHT EAR TORN																						
SEVERITIES	X			1	1	1	1	1	1	1	1	1										
TOTAL NO. OF OBS.				1	1	1	1	1	1	1	1	1										
MUCUS SECRETION FROM NOSE																						
SEVERITIES	X	18	33	8	2																	
TOTAL NO. OF OBS.		18	33	8	2																	
SIDES PINCHED IN																						
SEVERITIES	S		17	8	6			1														
	X			2																		
TOTAL NC OF OBS.			17	10	6			1														
STAINS AROUND NOSE																						
SEVERITIES	X	40	11	3	1	1																
TOTAL NO. OF OBS.		40	11	3	1	1																
UNGROOMED																						
SEVERITIES	X		22																			
TOTAL NO. OF OBS.			22																			
WET FUR																						
SEVERITIES	M	26																				
	S	14																				
TOTAL NO. OF OBS.		40																				

TABLE 4 - INTERGROUP COMPARISON OF CLINICAL OBSERVATIONS FOLLOWING EXPOSURE

90.1 mg/m3 — SEX: FEMALES

	DAY NUMBERS 22	23	24	25	26	27	28	29	30	31
KILLED TERMINATION										
SEVERITIES X										10
TOTAL NO. OF OBS.										10

GLOSSARY FOR STATISTICAL TABLES 5-8

Key to results of statistical tests:-

** Statistically significant difference from the control group mean at the 1% level (Student's t-test, two-sided).

* Statistically significant difference from the control group mean at the 5% level (Student's t-test, two-sided).

The nominal concentrations of 10,30 or 100mg/m^3 quoted on the Statistical Tables equate to the achieved concentrations of 9.9, 27 or 90.1mg/m^3 respectively

GLOSSARY FOR STATISTICAL TABLES 5-8

ADJUSTED MEAN

- Group mean bodyweight corrected for intergroup differences in group mean initial bodyweight. The adjusted means are calculated statistically using analysis of covariance. The size of the adjustment is determined by the size of the intergroup differences in group mean initial bodyweight and the strength of the relationship between initial bodyweight and subsequent bodyweights.

ORGAN WEIGHT ADJUSTED FOR BODYWEIGHT

- Group mean organ weight corrected for intergroup differences in group mean terminal bodyweight. The adjusted means are calculated statistically using analysis of covariance. The size of the adjustment is determined by the size of the intergroup differences in group mean terminal bodyweight and the strength of the relationship between organ weight and terminal bodyweight.

TABLE 5 - INTERGROUP COMPARISON OF BODYWEIGHTS (g)

DAY 2 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Day 1	MEAN	190.5	207.9	205.9	208.9
	S.D.	22.1	7.7	9.4	10.1
	N	10	10	10	10
Day 2	MEAN	193.7	203.5	187.4	183.5
	S.D.	19.0	7.5	6.6	7.7
	N	10	10	10	10
	ADJUSTED MEAN	204.3	199.7**	185.3**	178.9**

TABLE 5 - INTERGROUP COMPARISON OF BODYWEIGHTS (g)

DAY 4 KILL

		Concentration (mg/m**3)			
		0(Control)	10	30	100
Day 1	MEAN	199.4	201.3	202.3	202.1
	S.D.	7.1	8.3	7.6	6.6
	N	10	10	10	10
Day 4	MEAN	209.2	209.5	201.7	197.6
	S.D.	7.9	7.4	6.8	6.0
	N	10	10	10	10
	ADJUSTED MEAN	210.6	209.5	200.9**	197.0**

TABLE 5 - INTERGROUP COMPARISON OF BODYWEIGHTS (g)

DAY 11 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Day 1	MEAN	193.2	195.1	197.8	192.8
	S.D.	8.8	8.9	12.0	8.8
	N	10	10	10	10
Day 8	MEAN	220.5	221.5	216.9	212.7
	S.D.	12.9	15.7	10.9	10.7
	N	10	10	10	10
	ADJUSTED MEAN	222.0	221.1	213.8*	214.6*
Day 11	MEAN	227.4	233.3	229.7	224.9
	S.D.	10.5	15.8	9.1	12.0
	N	10	10	10	10
	ADJUSTED MEAN	228.6	233.0	227.2	226.5

TABLE 5 - INTERGROUP COMPARISON OF BODYWEIGHTS (g)

DAY 31 KILL

			Concentration (mg/m**3)			
			0 (Control)	10	30	100
Day 1		MEAN	191.2	192.6	192.4	194.5
		S.D.	7.9	7.3	11.2	8.0
		N	10	10	10	10
Day 8		MEAN	213.2	215.6	207.7	204.3
		S.D.	11.4	8.4	8.6	12.2
		N	10	10	10	10
	ADJUSTED	MEAN	214.4	215.7	207.9	202.8**
Day 15		MEAN	231.5	232.6	227.7	229.0
		S.D.	13.5	7.2	11.5	8.4
		N	10	10	10	10
	ADJUSTED	MEAN	232.5	232.6	227.9	227.8
Day 22		MEAN	245.8	246.9	244.3	246.9
		S.D.	13.2	8.2	12.6	8.5
		N	10	10	10	10
	ADJUSTED	MEAN	246.7	246.9	244.5	245.8
Day 29		MEAN	260.9	263.2	260.6	260.2
		S.D.	14.6	13.9	13.1	7.0
		N	10	10	10	10
	ADJUSTED	MEAN	261.6	263.2	260.7	259.3
Day 31		MEAN	264.7	267.3	263.6	262.7
		S.D.	16.4	14.8	14.8	10.7
		N	10	10	10	10
	ADJUSTED	MEAN	265.3	267.3	263.7	261.9

TABLE 6 - INTERGROUP COMPARISON OF LUNG WEIGHTS

DAY 2 KILL

		Concentration (mg/m**3) 0(Control)	10	30	100
Terminal Bodyweight (g)					
	MEAN	208.8	204.2	190.4	187.4
	S.D.	5.8	7.7	6.3	6.3
	N	5	5	5	5
Organ Weight (g)					
	MEAN	1.14	1.34	1.76**	2.20**
	S.D.	0.10	0.10	0.52	0.29
	N	5	5	5	5
Organ to Bodyweight Ratio (%)					
	MEAN	0.55	0.66	0.92	1.17
	S.D.	0.04	0.03	0.25	0.13
	N	5	5	5	5
Organ Weight Adjusted For Bodyweight		0.88	1.19	1.93**	2.44**

TABLE 6 - INTERGROUP COMPARISON OF LUNG WEIGHTS

DAY 4 KILL

		Concentration (mg/m**3) 0(Control)	10	30	100
Terminal Bodyweight (g)					
	MEAN	212.2	212.8	204.2	200.0
	S.D.	8.6	5.8	5.1	6.4
	N	5	5	5	5
Organ Weight (g)					
	MEAN	1.14	1.18	1.32**	1.61**
	S.D.	0.09	0.02	0.04	0.08
	N	5	5	5	5
Organ to Bodyweight Ratio (%)					
	MEAN	0.54	0.55	0.65	0.81
	S.D.	0.03	0.02	0.02	0.01
	N	5	5	5	5
Organ Weight Adjusted For Bodyweight		1.11	1.15	1.34**	1.66**

TABLE 6 - INTERGROUP COMPARISON OF LUNG WEIGHTS

DAY 11 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Terminal Bodyweight (g)					
	MEAN	232.2	241.4	229.8	223.8
	S.D.	10.0	15.9	9.7	12.1
	N	5	5	5	5
Organ Weight (g)					
	MEAN	1.20	1.27	1.30	1.40**
	S.D.	0.04	0.11	0.04	0.10
	N	5	5	5	5
Organ to Bodyweight Ratio (%)					
	MEAN	0.52	0.53	0.57	0.63
	S.D.	0.03	0.02	0.03	0.05
	N	5	5	5	5
Organ Weight Adjusted For Bodyweight		1.20	1.24	1.31*	1.43**

TABLE 6 - INTERGROUP COMPARISON OF LUNG WEIGHTS

DAY 31 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Terminal Bodyweight (g)					
	MEAN	268.0	268.0	268.6	264.8
	S.D.	14.9	0.0	18.6	7.6
	N	4	2	5	5
Organ Weight (g)					
	MEAN	1.23	1.32	1.27	1.29
	S.D.	0.02	0.18	0.06	0.07
	N	4	2	5	5
Organ to Bodyweight Ratio (%)					
	MEAN	0.46	0.49	0.47	0.49
	S.D.	0.03	0.07	0.03	0.02
	N	4	2	5	5
Organ Weight Adjusted For Bodyweight		1.23	1.31	1.27	1.29

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

DAY 2 KILL

		Concentration (mg/m**3)			
		0(Control)	10	30	100
Total Cell Count (x10**6/ml)					
	MEAN	3.32	3.72	5.42*	7.50**
	S.D.	0.87	1.18	1.87	1.33
	N	5	5	5	5
Alveolar Macrophage Count (x10**6/ml)					
	MEAN	3.30	2.23	2.02	1.60**
	S.D.	0.87	1.18	0.40	0.96
	N	5	5	5	5
Polymorphonuclear Leucocyte Count (x10**6/ml)					
	MEAN	0.01	1.37**	2.93**	5.82**
	S.D.	0.01	0.57	1.38	1.59
	N	5	5	5	5
Lymphocyte/Other Count (x10**6/ml)					
	MEAN	0.002	0.126**	0.473**	0.080
	S.D.	0.004	0.052	0.295	0.102
	N	5	5	5	5
Eosinophil Count (x10**6/ml)					
	MEAN	0.002	0.000	0.000	0.000
	S.D.	0.002	0.000	0.000	0.000
	N	5	5	5	5
Total Protein (mg/dl)					
	MEAN	23.5	140.8**	313.0**	705.9**
	S.D.	17.3	32.3	69.8	202.0
	N	5	5	5	5
Lactate Dehydrogenase (IU/l)					
	MEAN	23.6	65.0	13.2	87.8*
	S.D.	30.6	119.7	4.7	93.0
	N	5	5	5	5
Alkaline Phosphatase (IU/l)					
	MEAN	66.2	91.6	114.8*	167.8**
	S.D.	41.0	21.8	28.1	55.8
	N	5	5	5	5
N-acetyl glucosaminidase (IU/l)					
	MEAN	5.24	5.75	8.06*	11.35**
	S.D.	2.87	1.03	0.74	1.64
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

DAY 4 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Total Cell Count (x10**6/ml)					
	MEAN	1.72	5.16**	10.12**	19.24**
	S.D.	0 38	0.78	1.73	5.63
	N	5	5	5	5
Alveolar Macrophage Count (x10**6/ml)					
	MEAN	1.71	5.02**	9.00**	9.96**
	S.D.	0.38	0.74	1.72	2.41
	N	5	5	5	5
Polymorphonuclear Leucocyte Count (x10**6/ml)					
	MEAN	0.01	0.06	0.59**	4.60**
	S.D.	0.00	0.03	0.25	2.00
	N	5	5	5	5
Lymphocyte/Other Count (x10**6/ml)					
	MEAN	0.001	0.074	0.505**	4.685**
	S.D.	0.002	0.030	0.154	2.080
	N	5	5	5	5
Eosinophil Count (x10**6/ml)					
	MEAN	0.001	0.003	0.021	0.000
	S.D.	0.001	0.004	0.024	0.000
	N	5	5	5	5
Total Protein (mg/dl)					
	MEAN	12.3	21.0	17.4	66.8**
	S.D.	11.7	15.5	4.4	33.F
	N	5	5	5	5
Lactate Dehydrogenase (IU/l)					
	MEAN	60.0	184.6	192.2*	238.0**
	S.D.	11.9	242.3	117.2	65.8
	N	5	5	5	5
Alkaline Phosphatase (IU/l)					
	MEAN	41.4	47.0	56.8*	51.4
	S.D.	14.8	12.4	11.8	7.2
	N	5	5	5	5
N-acetyl glucosaminidase (IU/l)					
	MEAN	3.45	5.57	4.19	4.33
	S.D.	0.23	3.05	1.29	0.90
	N	2	3	3	4

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

DAY 11 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Total Cell Count (x10**6/ml)					
	MEAN	2.86	5.36	3.92	4.42
	S.D.	0.78	3.02	1.71	2.79
	N	5	5	5	5
Alveolar Macrophage Count (x10**6/ml)					
	MEAN	2.84	5.35	3.90	4.40
	S.D.	0.78	3.02	1.70	2.78
	N	5	5	5	5
Polymorphonuclear Leucocyte Count (x10**6/ml)					
	MEAN	0.01	0.00	0.00	0.00
	S.D.	0.00	0.00	0.00	0.00
	N	5	5	5	5
Lymphocyte/Other Count (x10**6/ml)					
	MEAN	0.014	0.008	0.018	0.014
	S.D.	0.008	0.004	0.015	0.011
	N	5	5	5	5
Eosinophil Count (x10**6/ml)					
	MEAN	0.000	0.001	0.000	0.000
	S.D.	0.000	0.001	0.000	0.000
	N	5	5	5	5
Total Protein (mg/dl)					
	MEAN	7.0	9.1	15.9	8.4
	S.D.	0.7	3.9	19.9	1.6
	N	5	5	5	5
Lactate Dehydrogenase (IU/l)					
	MEAN	8.0	5.8	6.4	5.0
	S.D.	2.1	2.2	3.2	2.2
	N	5	4	5	4
Alkaline Phosphatase (IU/l)					
	MEAN	54.6	46.0	44.0	42.0
	S.D.	13.3	14.3	10.1	14.2
	N	5	5	5	5
N-acetyl glucosaminidase (IU/l)					
	MEAN	3.82	4.44	4.01	4.14
	S.D.	0.29	1.41	0.79	0.54
	N	3	5	5	4

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

DAY 31 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Total Cell Count (x10**6/ml)					
	MEAN	0.50	0.42	0.45	0.56
	S.D.	0.20	0.16	0.09	0.13
	N	5	5	5	5
Alveolar Macrophage Count (x10**6/ml)					
	MEAN	0.50	0.42	0.44	0.55
	S.D.	0.19	0.16	0.09	0.13
	N	5	5	5	5
Polymorphonuclear Leucocyte Count (x10**6/ml)					
	MEAN	0.00	0.00	0.00	0.00
	S.D.	0.00	0.00	0.00	0.00
	N	5	5	5	5
Lymphocyte/Other Count (x10**6/ml)					
	MEAN	0.002	0.001	0.002	0.001
	S.D.	0.002	0.001	0.000	0.001
	N	5	5	5	5
Eosinophil Count (x10**6/ml)					
	MEAN	0.000	0.000	0.000	0.000
	S.D.	0.000	0.000	0.000	0.000
	N	5	5	5	5
Total Protein (mg/dl)					
	MEAN	17.1	8.1	8.6	7.1
	S.D.	15.5	4.7	5.4	3.4
	N	5	5	5	5
Lactate Dehydrogenase (IU/l)					
	MEAN	228.2	95.2	104.4	73.6
	S.D.	241.5	43.4	41.8	46.6
	N	5	5	5	5
Alkaline Phosphatase (IU/l)					
	MEAN	60.2	51.6	49.2	39.8*
	S.D.	15.6	5.5	13.4	10.2
	N	5	5	5	5
N-acatyl glucosaminidase (IU/l)					
	MEAN	7.18	4.69	3.53	3.43
	S.D.	2.62	0.99		
	N	2	3	1	1

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3) 0 (Control)	10	30	100
Total Cell Count (x10**6/ml)					
Day 2	MEAN	3.32	3.72	5.42*	7.50**
	S.D.	0.87	1.18	1.87	1.33
	N	5	5	5	5
Day 4	MEAN	1.72	5.16**	10.12**	19.24**
	S.D.	0.38	0.78	1.73	5.63
	N	5	5	5	5
Day 11	MEAN	2.86	5.36	3.92	4.42
	S.D.	0.78	3.02	1.71	2.79
	N	5	5	5	5
Day 31	MEAN	0.50	0.42	0.45	0.56
	S.D.	0.20	0.16	0.09	0.13
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3) 0(Control)	10	30	100
Alveolar Macrophage Count (x10**6/ml)					
Day 2	MEAN	3.30	2.23	2.02	1.60**
	S.D.	0.87	1.18	0.40	0.96
	N	5	5	5	5
Day 4	MEAN	1.71	5.02**	9.60**	9.96**
	S.D.	0.38	0.74	1.72	2.41
	N	5	5	5	5
Day 11	MEAN	2.84	5.35	3.90	4.40
	S.D.	0.78	3.02	1.70	2.78
	N	5	5	5	5
Day 31	MEAN	0.50	0.42	0.44	0.55
	S.D.	0.19	0.16	0.09	0.13
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3) 0(Control)	10	30	100
Polymorphonuclear Leucocyte Count (x10**6/ml)					
Day 2	MEAN	0.01	1.37**	2.93**	5.82**
	S.D.	0.01	0.57	1.38	1.59
	N	5	5	5	5
Day 4	MEAN	0.01	0.06	0.59**	4.60**
	S.D.	0.00	0.03	0.25	2.00
	N	5	5	5	5
Day 11	MEAN	0.01	0.00	0.00	0.00
	S.D.	0.00	0.00	0.00	0.00
	N	5	5	5	5
Day 31	MEAN	0.00	0.00	0.00	0.00
	S.D.	0.00	0.00	0.00	0.00
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3)			
		0 (Control)	10	30	100
Lymphocyte/Other Count (x10**6/ml)					
Day 2	MEAN	0.002	0.126**	0.473**	0.080
	S.D.	0.004	0.052	0.295	0.102
	N	5	5	5	5
Day 4	MEAN	0.001	0.074	0.505**	4.685**
	S.D.	0.002	0.030	0.154	2.080
	N	5	5	5	5
Day 11	MEAN	0.014	0.008	0.018	0.014
	S.D.	0.008	0.004	0.015	0.011
	N	5	5	5	5
Day 31	MEAN	0.002	0.001	0.002	0.001
	S.D.	0.002	0.001	0.000	0.001
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3) 0 (Control)	10	30	100
Eosinophil Count (x10**6/ml)					
Day 2	MEAN	0.002	0.000	0.000	0.000
	S.D.	0.002	0.000	0.000	0.000
	N	5	5	5	5
Day 4	MEAN	0.001	0.003	0.021	0.000
	S.D.	0.001	0.004	0.024	0.000
	N	5	5	5	5
Day 11	MEAN	0.000	0.001	0.000	0.000
	S.D.	0.000	0.001	0.000	0.000
	N	5	5	5	5
Day 31	MEAN	0.000	0.000	0.000	0.000
	S.D.	0.000	0.000	0.000	0.000
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3) 0 (Control)	10	30	100
Total Protein (mg/dl)					
Day 2	MEAN	23.5	140.8**	313.0**	705.9**
	S.D.	17.3	32.3	69.8	202.0
	N	5	5	5	5
Day 4	MEAN	12.3	21.0	17.4	66.8**
	S.D.	11.7	15.5	4.4	33.5
	N	5	5	5	5
Day 11	MEAN	7.0	9.1	15.9	8.4
	S.D.	0.7	3.9	19.9	1.6
	N	5	5	5	5
Day 31	MEAN	17.1	8.1	8.6	7.4
	S.D.	15.5	4.7	5.4	3.4
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3) 0(Control)	10	30	100
Lactate Dehydrogenase (IU/l)					
Day 2	MEAN	23.6	65.0	13.2	87.8*
	S.D.	30.6	119.7	4.7	93.0
	N	5	5	5	5
Day 4	MEAN	60.0	184.6	192.2*	238.0**
	S.D.	11.9	242.3	117.2	65.8
	N	5	5	5	5
Day 11	MEAN	8.0	5.8	6.4	5.0
	S.D.	2.1	2.2	3.2	2.2
	N	5	4	5	4
Day 31	MEAN	228.2	95.2	104.4	73.6
	S.D.	241.5	43.4	41.8	46.6
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3)			
		0 (Control)	10	30	100
Alkaline Phosphatase (IU/l)					
Day 2	MEAN	66.2	91.6	114.8*	167.8**
	S.D.	41.0	21.8	28.1	55.8
	N	5	5	5	5
Day 4	MEAN	41.4	47.0	56.8*	51.4
	S.D.	14.8	12.4	11.8	7.2
	N	5	5	5	5
Day 11	MEAN	54.6	46.0	44.0	42.0
	S.D.	13.3	14.3	10.1	14.2
	N	5	5	5	5
Day 31	MEAN	60.2	51.6	49.2	39.8*
	S.D.	15.6	5.5	13.4	10.2
	N	5	5	5	5

TABLE 7 - INTERGROUP COMPARISON OF LUNG LAVAGE FLUID

		Concentration (mg/m**3) 0(Control)	10	30	100
N-acetyl glucosaminidase (IU/l)					
Day 2	MEAN	5.24	5.75	8.06*	11.35**
	S.D.	2.87	1.03	0.74	1.64
	N	5	5	5	5
Day 4	MEAN	3.45	5.57	4.19	4.33
	S.D.	0.23	3.05	1.29	0.90
	N	2	3	3	4
Day 11	MEAN	3.82	4.44	4.01	4.14
	S.D.	0.29	1.41	0.79	0.54
	N	3	5	5	4
Day 31	MEAN	7.18	4.69	3.53	3.43
	S.D.	2.62	0.99		
	N	2	3	1	1

TABLE 8 - INTERGROUP COMPARISON OF BrdU LABELLING INDICES

DAY 2 KILL

		Concentration (mg/m**3) 0(Control)	10	30	100
Terminal Bronchioles (%)	MEAN	5.1	6.9	9.2	6.7
	S.D.	3.2	1.9	4.1	4.9
	N	5	5	5	5
Centro-acinar alveoli (%)	MEAN	4.6	8.8	8.7	6.1
	S.D.	2.2	5.3	1.8	2.8
	N	5	5	5	5

TABLE 8 - INTERGROUP COMPARISON OF BrdU LABELLING INDICES

DAY 4 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Terminal Bronchioles (%)	MEAN	3.7	13.9*	26.0**	42.5**
	S.D.	1.1	4.8	11.8	10.4
	N	3	5	5	5
Centro-acinar alveoli (%)	MEAN	6.7	11.6	22.3**	45.0**
	S.D.	2.4	3.6	7.7	13.3
	N	3	5	5	5

TABLE 8 - INTERGROUP COMPARISON OF BrdU LABELLING INDICES

DAY 11 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Terminal Bronchioles (%)	MEAN	5.5	5.7	11.1*	8.8
	S.D.	1.2	1.4	6.4	2.1
	N	5	5	5	5
Centro-acinar alveoli (%)	MEAN	4.8	5.3	9.4*	7.8
	S.D.	2.2	0.9	5.9	2.8
	N	5	5	5	5

TABLE 8 - INTERGROUP COMPARISON OF BrdU LABELLING INDICES

DAY 31 KILL

		Concentration (mg/m**3) 0 (Control)	10	30	100
Terminal Bronchioles (%)	MEAN	4.0	3.5	2.5	3.7
	S.D.	1.0	0.6	1.0	1.6
	N	5	5	4	5
Centro-acinar alveoli (%)	MEAN	3.4	3.0	3.2	4.2
	S.D.	1.3	0.9	0.6	1.2
	N	5	5	4	5

TABLE 8 - INTERGROUP COMPARISON OF BrdU LABELLING INDICES

		Concentration (mg/m**3) 0(Control)	10	30	100
Terminal Bronchioles (%)					
Day 2	MEAN	5.1	6.9	9.2	6.7
	S.D.	3.2	1.9	4.1	4.9
	N	5	5	5	5
Day 4	MEAN	3.7	13.9*	26.0**	42.5**
	S.D.	1.1	4.8	11.8	10.4
	N	3	5	5	5
Day 11	MEAN	5.5	5.7	11.1*	8.8
	S.D.	1.2	1.4	6.4	2.1
	N	5	5	5	5
Day 31	MEAN	4.0	3.5	2.5	3.7
	S.D.	1.0	0.6	1.0	1.6
	N	5	5	4	5

TABLE 8 - INTERGROUP COMPARISON OF BrdU LABELLING INDICES

		Concentration (mg/m**3)			
		0(Control)	10	30	100
Centro-acinar alveoli (%)					
Day 2	MEAN	4.6	8.8	8.7	6.1
	S.D.	2.2	5.3	1.8	2.8
	N	5	5	5	5
Day 4	MEAN	6.7	11.6	22.3**	45.0**
	S.D.	2.4	3.6	7.7	13.3
	N	3	5	5	5
Day 11	MEAN	4.8	5.3	9.4*	7.8
	S.D.	2.2	0.9	5.9	2.8
	N	5	5	5	5
Day 31	MEAN	3.4	3.0	3.2	4.2
	S.D.	1.3	0.9	0.6	1.2
	N	5	5	4	5

GLOSSARY FOR TABLES 9 AND 11

Key:

NO	number
Grp	group

TABLE 9 - INTERGROUP COMPARISON OF MACROSCOPIC FINDINGS

SEX: FEMALES	0 mg/m3	9.9 mg/m3	27.0 mg/m3	90.1 mg/m3
FEMALES ON STUDY	40	40	40	40
ANIMALS COMPLETED	20	20	20	20
LUNG				
SUBMITTED	20	20	20	20
NO.WITH FINDINGS	0	4	5	5
NO.WITHOUT FINDINGS	20	16	15	15
Pale spot/s or area/s	0	4	5	5
Raised area/s	0	2	5	5
THYMUS				
NO.WITH FINDINGS BUT NOT SUBMITTED	0	1	0	0
Speckled	0	1	0	0

TABLE 10 - INTERGROUP COMPARISON OF MICROSCOPIC FINDINGS

NON-NEOPLASTIC MORPHOLOGIES SEX: FEMALES	0 mg/m3	9.9 mg/m3	27.0 mg/m3	90.1 mg/m3
FEMALES ON STUDY	40	40	40	40
ANIMALS COMPLETED	20	20	20	20
LUNG				
EXAMINED	20	20	20	20
NO ABNORMALITIES DETECTED	18	6	5	5
Alveolar macrophage infiltration (TOTAL)	0	10	10	12
minimal	0	9	6	2
slight	0	1	4	7
moderate	0	0	0	3
Pneumonitis (TOTAL)	0	6	5	10
minimal	0	5	1	2
slight	0	1	4	6
moderate	0	0	0	2
Intra-bronchiolar cell exudate (TOTAL)	0	2	3	7
minimal	0	1	0	3
slight	0	1	1	3
moderate	0	0	2	1
Airway proteinaceous effusion (TOTAL)	0	5	4	5
minimal	0	3	2	0
slight	0	2	0	2
moderate	0	0	1	2
marked	0	0	1	1
Bronchiolar epithelial hypertrophy/hyperplasia (TOTAL)	0	2	4	4
minimal	0	1	0	0
slight	0	1	4	2
moderate	0	0	0	2
Alveolar epithelialization (TOTAL)	0	4	9	8
minimal	0	3	5	1
slight	0	1	4	7
Thickening - alveolar duct wall (TOTAL)	1	3	5	4
minimal	1	3	3	4
slight	0	0	2	0
Emphysematous change (TOTAL)	0	0	0	1
minimal	0	0	0	1

TABLE 10 - INTERGROUP COMPARISON OF MICROSCOPIC FINDINGS

NON-NEOPLASTIC MORPHOLOGIES	SEX: FEMALES	0 mg/m3	9.9 mg/m3	27.0 mg/m3	90.1 mg/m3
	FEMALES ON STUDY	40	40	40	40
	ANIMALS COMPLETED	20	20	20	20
LUNG	(CONTINUED)				
Squamous cyst		1	0	0	0

TABLE 11 - ULTRASTRUCTURAL CHANGES IN THE LUNGS OF RATS EXPOSED TO POLYMERIC MDI

Day 2

Grp	Dose (mg/m^3)	Type II	Type II Lamellar Bodies		Macrophage			Lumen	
		Hyperplasia	Increased No.	Increased size	Surfactant		Amorphous Material	Crystalline	Debris
					Lamellar	Crystalline			
1.1	0								
1.2	9.9					+		++	++
1.3	27				+	+		++	++
1.4	90.1			+	++	+	+	++	+++

Key: + = Minimal, ++ = Slight, +++ = Moderate, ++++ = Marked

TABLE 11 - ULTRASTRUCTURAL CHANGES IN THE LUNGS OF RATS EXPOSED TO POLYMERIC MDI

Day 4

Grp	Dose (mg/m^3)	Type II	Type II lamellar bodies		Macrophage			Lumen	
		Hyperplasia	Increased No	Increased size	Surfactant		Amorphous	Crystalline	Debris
					Lamellar	Crystalline			
2.1	0				+	+			
2.2	9.9	+	+		++	+		+	
2.3	27	++			+++	+	+	+	
2.4	90.1	++/+++	+++	+++	++++	++	+	++/+++	+++

Key: + = Minimal, ++ = Slight, +++ = Moderate, ++++ = Marked

TABLE 11 - ULTRASTRUCTURAL CHANGES IN THE LUNGS OF RATS EXPOSED TO POLYMERIC MDI

Day 11

Grp	Dose (mg/m^3)	Type II	Type II lamellar bodies		Macrophage			Lumen	
		Hyperplasia	Increased No.	Increased size	Surfactant		Amorphous Material	Crystalline	Debris
					Lamellar	Crystalline			
3.1	0								
3.2	9.9	+			+				+
3.3	27				+	+		+	+
3.4	90.1	+		++	++				+

Key: + = Minimal, ++ = Slight, +++ = Moderate, ++++ = Marked

TABLE 11 - ULTRASTRUCTURAL CHANGES IN THE LUNGS OF RATS EXPOSED TO POLYMERIC MDI

Day 31

Grp	Dose (mg/m^3)	Type II	Type II Lamellar bodies		Macrophage			Lumen	
		Hyperplasia	Increased No.	Increased size	Surfactant		Amorphous material	Crystalline	Debris
					Lamellar	Crystalline			
4.1	0							+	
4.2	9.9				+				
4.3	27				+				
4.4	90.1				+			+	

Key: +, = Minimal, ++ = Slight, +++ = Moderate, ++++ = Marked

APPENDIX A - THE DETERMINATION OF POLYMERIC MDI IN SAMPLES TAKEN FOR TOTAL PARTICULATE

Method summary

The test substance was extracted/desorbed from filters with a known volume of acetic anhydride in acetonitrile. After appropriate derivatisation and dilution an aliquot was removed. The samples and standards were then analysed by High Performance Liquid Chromatography (HPLC).

Chemicals and reagents

Acetonitrile, HPLC grade

Water, Milli Q+ grade (Millipore)

Glacial acetic acid, analytical grade

Sodium acetate, analytical grade

Toluene, HPLC grade

Acetic anhydride, analytical grade

Diethyl phthalate, analytical grade

1-(2-methoxyphenyl)piperazine, analytical grade

Derivatisation

Derivatising Solution

Isocyanate samples were derivatised using 1-(2-methoxyphenyl)piperazine (1,2-MP).

Approximately 400mg of 1,2-MP was accurately weighed into a 10ml volumetric flask and 500mg diethyl phthalate added. This was made to volume with toluene and designated Solution A, for use in coating filters. Approximately 50mg of 1,2-MP was accurately weighed into a 100ml volumetric flask and made to volume with toluene to produce a stock 1,2-MP solution. This stock solution was diluted to make 260µmolar solution which was used to complete the derivatisation of the filters.

APPENDIX A - THE DETERMINATION OF POLYMERIC MDI IN SAMPLES TAKEN FOR TOTAL PARTICULATE

Filter preparation

Solution A (2ml) and toluene (5.25ml) were mixed and 200µl of the resultant solution was used to coat each filter. Then the filter was placed in the dark to dry and sealed in a vial for storage.

Calibration standards

Analytical standard

Polymeric MDI (Suprasec 5005), CTL Reference Y00122/021, with an assumed purity of 80% w/w was used.

Varying amounts of Polymeric MDI was accurately weighed into a volumetric flask and diluted to volume with toluene to give a stock standard of known concentration. (e.g. 157.5mg Polymeric MDI made to 100ml with toluene to give a stock solution then diluted to give working standards, after derivatisation.)

Further appropriate dilutions were made with toluene in volumetric flasks to give a range of solutions, within 0.59µg/ml to 2.78µg/ml Di-MDI and 0.31µg/ml to 1.45µg/ml Tri-MDI.

The purity of the test substance was taken into account in the preparation of the standard solutions.

Procedure

Sample preparation

A known volume (2ml) of 260µmolar 1,2-MP solution was added to each filter in the container provided with the sample. The vial was sealed and left for 24 hours in darkness. Acetic anhydride (100µl) was then added to the vial and the whole sample was evaporated to dryness under nitrogen.

APPENDIX A - THE DETERMINATION OF POLYMERIC MDI IN SAMPLES TAKEN FOR TOTAL PARTICULATE

A known volume of acetonitrile (2ml) was added and gently agitated in the vial. The filter and solution were then decanted into UNIPREP syringeless filter and filtered into a clean vial. An aliquot was then removed for subsequent analysis.

High Performance Liquid Chromatography Conditions

Modular system	:	LC Module 1 (Waters)
Comprising		
Pump	:	600 Series (Waters)
Detectors	:	484 UV detector(Waters)
	:	464 electrochemical detector (Waters)
Mobile phase	:	acetonitrile (60% v/v)
		0.5% sodium acetate buffer pH 6 (40% v/v)
Flow rate	:	1ml/min
Detector wavelength	:	242nm
464 Electrochemical		
detector settings	:	Detector mode : DC
	:	Working electrode : Glassy carbon
	:	Reference electrode : Ag / AgCl
	:	Potential : +800mV
	:	Current range : 0.2µA
Column	:	25cm x 4.6mm ID S5ODS2 (HiChrom)
Column temperature	:	Ambient
Injection volume	:	25µl
Data handling	:	Waters 860 Data System (Waters)

Calibration

The analysis system was calibrated using a range of standards to determine the linearity of response.

APPENDIX A - THE DETERMINATION OF POLYMERIC MDI IN SAMPLES TAKEN FOR TOTAL PARTICULATE

Calculation of results

Total Particulate Samples

Analysed atmosphere concentration (µg/l) $Ca = \frac{Cs \times Df}{Va \times 1000}$

Where Cs = Calculated sample concentration from data system (µg/ml)

Df = Dilution factor

Va = Atmosphere sample volume (l)

= Sample collection time (min) x flow (l/min)

$\%\ \text{Total Particulate} = \frac{Ca \times 100}{Cg}$

Where Cg = Gravimetric atmosphere concentration

Limit of detection

The limit of detection was calculated to be approximately 0.03µg/ml Di-MDI and 0.04µg/ml Tri-MDI in the analysed solution, corresponding to an atmosphere concentration of 0.07µg/l Di-MDI and 0.10µg/l Tri-MDI.

APPENDIX B - PARTICLE SIZE CLASSIFICA . N

1. Mass median aerodynamic diameter (MMAD)

The mass median aerodynamic diameter (MMAD) of an aerosol, or part of an aerosol, is the diameter of a unit density sphere having the same terminal settling velocity as a particle shown to divide the size distribution of the aerosol in half when measured by mass. The algebraic symbol commonly used for the MMAD is "D_{50}".

2. Geometric standard deviation (GSD)

The GSD of an aerosol is the ratio of the mean of the distribution to the mean ± 1 standard deviation ie:

$$GSD\ (\delta g) = \frac{D50}{D16} = \frac{D84}{D50} = \sqrt{\frac{D84}{D16}}$$

This relationship is only valid for aerosols with a log normal distribution, which is considered to be the case in this study.

APPENDIX C - ALLOCATION OF RATS TO EXPERIMENTAL GROUPS

The animals were distributed amongst experimental groups by a method designed to ensure that any unhealthy rats or rats at the extremes of the weight range were excluded.

The weights were sorted using a computer sort feature. Clinical observations were recorded if present. All the stock animals were weighed and the data recorded directly electronically. The weights were then sorted electronically into order with the highest weight listed first, followed by the next highest etc. Animals at extremes of the weight range or with clinical findings were discarded.

A Latin Square was generated and was used for the allocation of animals to the experimental groups. The heaviest animal was allocated the lowest number in the group of replicate 1 denoted by the number on the Latin Square and ear-punched with the lowest available experimental number for that cage shown on the rack plan. This procedure was carried out until all cages in each replicate contained one rat.

The procedure was then repeated until each cage contained five rats.

APPENDIX D - ARRANGEMENT OF ANIMALS ON THE RACKS SHOWING INDIVIDUAL ANIMAL NUMBERS AND POSITIONS

Rep	Rack 1			
1	1-5 (1)	11-15 (2)	31-35 (4)	21-25 (3)
2	16-20 (2)	6-10 (1)	26-30 (3)	36-40 (4)
3	101-105 (1)	111-115 (2)	121-125 (3)	131-135 (4)
4	126-130 (3)	106-110 (1)	136-140 (4)	116-120 (2)

Rep	Rack 2			
5	211-215 (2)	221-225 (3)	201-215 (1)	231-135 (4)
6	206-210 (1)	236-240 (4)	216-220 (2)	226-230 (3)
7	331-335 (4)	321-325 (3)	311-315 (2)	301-305 (1)
8	316-320 (2)	306-310 (1)	336-340 (4)	326-330 (3)

APPENDIX E - METHOD OF NOSE ONLY EXPOSURE AND RESTRAINT

1. Purpose

The nose-only method of exposure was used since deposition on the fur of the test particulate and subsequent ingestion during grooming can result in substantial quantities of test material entering the body by the oral route, exacerbating and possibly masking any effects produced by inhalation.

2. Design (Figure 1)

2.1 The chamber

The chamber consisted of sections of PERSPEX tubing (6mm wall thickness) with an internal diameter of 28cm and height of 15cm (approximate volume 9.2 litres). Each section was drilled with ten equidistant holes of 28mm diameter into which the restraining tubes were pushed to give a good seal. There was also one sampling port.

The chamber located on to a base-plate, fitted with castors for manoeuvrability. A conical aluminium lid ensured good distribution of the atmosphere across the chamber, the atmosphere having being generated from above. The conical lid and the base together had a volume of approximately 9.2 litres. In this study two sections were connected, giving a volume of approximately 27.6 litres.

APPENDIX E - METHOD OF NOSE ONLY EXPOSURE AND RESTRAINT

2.2 The restraining tubes

The restraining tubes were made by Battelle, Geneva and consisted of a polycarbonate tube, one end of which was tapered and fitted into the exposure chamber. In the other end a spring loaded plunger was fitted which was contoured to fit a rat and fitted over the base of the tail. The top of the tube had apertures to prevent excessive build-up of heat and water vapour which might make the animal unduly uncomfortable, while similarly there was a groove in the bottom of the tube for drainage of urine.

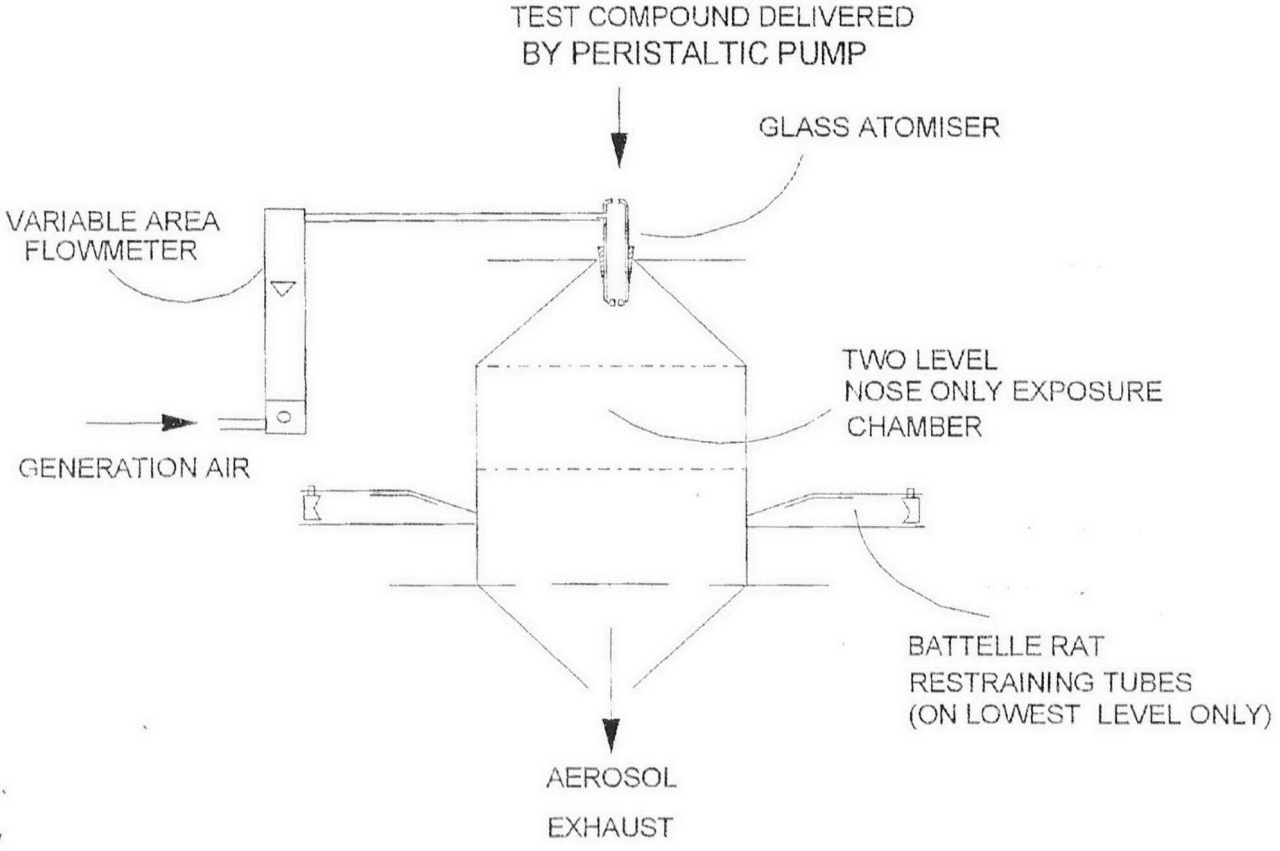
3. Sampling

Atmospheric concentrations were determined by sampling through the sampling port. While atmospheres were being set up prior to animal exposure the holes for the restraining tubes were plugged.

APPENDIX E - METHOD OF NOSE ONLY EXPOSURE AND RESTRAINT

FIGURE 1

DIAGRAM OF 2-TIERED EXPOSURE CHAMBER
WITH CONCENTRIC GLASS JET ATOMISER



APPENDIX F - GROUP RANGE DAILY CHAMBER TEMPERATURES AND RELATIVE HUMIDITY

These data were taken from the daily readings of temperature and relative humidity recorded during the exposure period in the exposure chamber.

Day no 1.	Date	Temperature (°C)	Relative Humidity (%)
Group 1	17/11/97	18.5-20	36-47
Group 2	17/11/97	18.7-20	37-46
Group 3	17/11/97	18.8-20	31-44
Group 4	17/11/97	18-19.9	12-27
Day no 2			
Group 1	25/11/97	17.7-19.3	24-33
Group 2	25/11/97	17.7-20	17-25
Group 3	25/11/97	17.5-20	19-33
Group 4	25/11/97	17.7-20.1	15-23
Day no 3			
Group 1	2/12/98	18.5-19.4	12-18
Group 2	2/12/98	18.6-19.6	12-15
Group 3	2/12/98	18.1-19.6	9-12
Group 4	2/12/98	18.5-19.7	11-15

APPENDIX G - DETAILS OF STATISTICAL METHODS

Bodyweights were considered by analysis of covariance on initial (day 1) bodyweight.

Lung weights were considered by analysis of variance and analysis of covariance on final bodyweight. Summary statistics are presented for lung to bodyweight ratios but these were not analysed statistically as the analysis of covariance provides a better method of allowing for differences in terminal bodyweights (Shirley, 1996).

Brdu labelling indices were considered by analysis of variance following a double arcsine transformation (Freeman and Tukey, 1950).

Lung lavage fluid data were considered by analysis of variance. Total protein, alkaline phosphatase and lactate dehydrogenase were transformed before analysis using a natural logarithmic transformation. Total cell counts, alveolar macrophage counts, polymorphonuclear leucocyte counts and lymphocyte/other counts were transformed before analysis using a square root transformation. Eosinophil counts were not analysed statistically as most values were zero.

All analyses were carried out separately by day of kill.

Analyses of variance and covariance were carried out using the GLM procedure in SAS(1989). Analyses of bodyweight allowed for the replicate structure of the study design. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

APPENDIX G - DETAILS OF STATISTICAL METHODS

Data excluded from statistical analysis

For lactate dehydrogenase and n-acetyl glucosaminidase all missing values were recorded as below the limit of detection (3). The omission of these values has not affected the interpretation. Analyses allowing for left-censoring could be carried out if required but they are more complicated and would not be of major benefit here.

Adjusted mean:

- Group mean bodyweight corrected for intergroup differences in group mean initial bodyweight. The adjusted means are calculated statistically using analysis of covariance. The size of the adjustment is determined by the size of the intergroup differences in group mean initial bodyweight and the strength of the relationship between initial bodyweight and subsequent bodyweights.

Organ weight adjusted for bodyweight:

- Group mean organ weight corrected for intergroup differences in group mean terminal bodyweight. The adjusted means are calculated statistically using analysis of covariance. The size of the adjustment is determined by the size of the intergroup differences in group mean terminal bodyweight and the strength of the relationship between organ weight and terminal bodyweight (Shirley E 1996).

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